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Obesity and cancer: Update on etiology, molecular biomarkers and biotargets, clinical strategies, and epidemiology

Edited by
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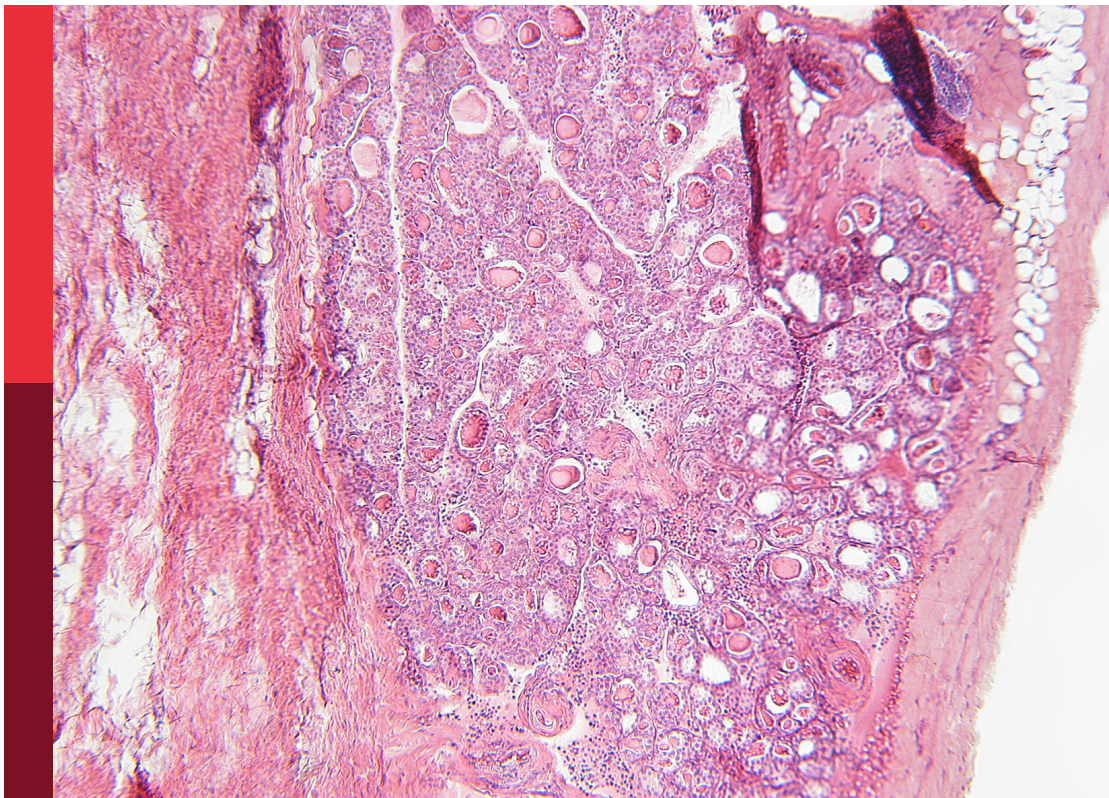

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Obesity and cancer: Update on etiology, molecular biomarkers and biotargets, clinical strategies, and epidemiology

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Editorial: Obesity and cancer: update on etiology, molecular biomarkers and biotargets, clinical strategies, and epidemiology

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KEYWORDS

obesity, cancer, animal models, epidemiology—analytic (risk factors), global prevalence

Editorial on the Research Topic
Obesity and cancer: update on etiology, molecular biomarkers and biotargets, clinical strategies, and epidemiology

Obesity continues to evolve as a significant global health crisis. It is estimated that by 2025, 2.7 billion adults will be overweight, over 1 billion will be obese, and 177 million will be extremely obese (1, 2). In addition to severe comorbidities like diabetes and cardiovascular disease, the most recent data prove that obesity is associated with developing as many as 18 types of cancer (1, 3). Furthermore, excess body weight increases cancer survivors' risk of disease recurrence and mortality (4).

Even though human and animal studies validated the link between obesity and cancer, the underlying mechanisms are not entirely understood. At present, vast experimental and evolving clinical evidence links the occurrence of cancer in obese and overweight patients to processes such as chronic inflammation, altered fatty acid metabolism, insulin resistance, abnormal activation of adipokines and anabolic and sex hormones, extracellular matrix remodeling, microbial dysbiosis (1, 4). Nevertheless, new biological pathways are continuously being uncovered and could lead to new perspectives and targets for therapies

In addition to mechanistic considerations, a better understanding of sociological and regional differences in obesity prevalence and cancer risks would be critical in devising the most promising prevention and intervention strategies (5).

This Research Topic includes several reviews and original papers providing updated perspectives on the associations between obesity and cancer. The contributions detail new biomarkers and biotargets, prospective interventions and treatments, and epidemiologic analyses.

Obesity is often associated with metabolic syndrome and diabetes mellitus. Accordingly, several papers in this Research Topic address the link between these pathologies and cancer.

Sun et al. provide new information on the association between prediabetes and diabetes status and breast cancer based on the US National Health and Nutrition Examination Survey. They report that diabetes mellitus is associated with the risk of breast cancer development, and the risk of developing breast cancer increases steadily from non-diabetes to prediabetes and type 2 diabetes. Furthermore, age has a threshold effect on the risk of breast cancer in females, with the risk increasing significantly after age 52.

A systematic review and meta-analysis by Lu and Tao report that diabetes (type 2) and obesity are risk factors for bladder cancer prognosis. The meta-analysis suggests that both diabetes and excessive body weight can negatively influence bladder cancer outcomes such as mortality, progression, and recurrence. However, the risk of mortality due to diabetes in patients with bladder cancer was similar to that in the general population.

Insulin resistance and inflammation have also been shown by Li et al. to be critical mediators of abdominal obesity-related colorectal cancer (CRC) risk. The study reports that C-reactive protein (CRP) and the fasting triglyceride-glucose (TyG) index increased the risk of colorectal cancer independently and synergistically. CRP and the TyG are also reported as mediators for the association between abdominal obesity and CRC risk. These parameters may help clarify the role of abdominal fat disposition over overall obesity in CRC.

A systematic meta-analysis by Zhong et al. suggests a significant relationship between metabolic syndrome and pancreatic cancer. Patients with metabolic syndrome were more likely to develop pancreatic cancer, regardless of gender. The increased risk of developing pancreatic cancer was strongly linked to hypertension, poor high-density lipoprotein cholesterol ratio, and hyperglycemia. However, the prevalence of pancreatic cancer was independent of obesity and hypertriglyceridemia.

This Research Topic also addresses the less well-known relationships between obesity and cancer. For example, the review from Marques-Mourlet et al. examines the clinical and mechanistic impact of obesity on the progression of multiple myeloma (MM). They describe the currently available models for studying obesity in mouse myeloma models and summarize what is known in the field regarding the role of obesity in MM based on epidemiological and preclinical research demonstrating that obesity increases the risk for MM but that the "obesity paradox" persists in terms of outcomes, where obesity does not consistently correlate with worse outcomes.

Chen et al. discuss the links between obesity, non-alcoholic fatty liver disease, and hepatocellular carcinoma. The review focuses on molecular mechanisms and cellular signaling pathways involved in the pathogenesis of obesity-associated hepatocellular carcinoma. The authors also summarize the preclinical, experimental animal models and the non-invasive diagnostic methods of non-alcoholic fatty liver disease and hepatocellular carcinoma and discuss novel therapies for hepatocellular carcinoma in obese patients.

Feng et al. provide novel data on overweight-related transcriptomic signature as a marker for treatment response in hepatocellular carcinoma. Notably, the authors report that the overweight/obesity-associated gene (OAG) signature, including 17 genes, provides reliable performance in the prognosis prediction of hepatocellular carcinoma.

Llanos et al. focus on the molecular mechanisms involved in the association between overall and central body fatness and poorer breast cancer outcomes. The study reports altered gene and/or protein expression of the obesity hormone leptin and its receptor in this process. The authors report that increased body fatness is associated with increased leptin gene expression and elevated leptin receptor levels in breast tumors.

Finally, Zhang et al. review animal models to study obesity and cancer associations. The authors argue that replicating both obesity and malignancy in laboratory animals is extremely difficult. Animals commonly used in obesity research cannot engraft heterolytic tumors. On the other hand, it is challenging to induce obesity in animals commonly used as cancer models. This review summarizes several experimental animal models and protocols that can simultaneously generate obesity and sustain the growth of tumor xenografts.

What is the future of targeting obesity-related cancer? In preventing obesity-associated cancers, weight-reducing strategies such as structured exercise in combination with dietary support and behavior therapy will continue to be the mainstay of interventions. Treatment with glucagon-like peptide-1 analogs and bariatric surgery that produce significant and rapid weight loss might become preventive options in some individuals, such as high-risk patients or selected cancer survivors (1). The discovery of specific obesity-related pathways common for different neoplasms might offer an additional treatment option.

Author contributions

ES: Conceptualization, Writing – original draft, Writing – review & editing. VG: Writing – original draft, Writing – review & editing. MR: Writing – original draft, Writing – review & editing.

Conflict of interest

Author ES was employed by the company Allysta Pharmaceuticals, Inc. This relationship did not influence in any way the content of the article.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
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Diabetes Mellitus and Obesity as Risk Factors for Bladder Cancer Prognosis: A Systematic Review and Meta-Analysis

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Background: Urinary bladder carcinoma is common in developed settings, and prognosis may be impacted by lifestyle factors such as excess body weight and diabetes mellitus. The present meta-analysis aimed to systematically collate and analyze evidence on the impact of diabetes and excess BMI on bladder cancer outcomes.

Methods: PubMed, Scopus, and Google Scholar databases were screened for relevant studies that examined the association between bladder cancer outcomes and diabetes and/or excess body weight. The primary outcomes for this study were mortality (both all-cause and cancer-specific), risk of cancer progression, and recurrence. Strength of association was presented in the form of pooled adjusted hazard ratios (HR). Statistical analysis was performed using STATA version 16.0.

Results: Twenty-five articles met inclusion criteria. Nine of these examined diabetes mellitus while 16 studied body mass index. All studies were retrospective. Diabetic patients had significantly higher risk for all-cause mortality (HR 1.24, 95% CI: 1.07, 1.44, n=3), cancer specific mortality (HR 1.67, 95% CI: 1.29, 2.16, n=7), disease progression (HR 1.54, 95% CI: 1.15, 2.06, n=8), and recurrence (HR 1.40, 95% CI: 1.32, 1.48, n=8) compared to non-diabetics. No statistically significant risk change for all-cause mortality, cancer specific mortality, disease progression, and recurrence was found for overweight patients. However, obese individuals were at higher risk for disease progression (HR 1.88, 95% CI: 1.41, 2.50, n=3) and recurrence (HR 1.60, 95% CI: 1.06, 2.40, n=7) compared to normal BMI patients.

Conclusions: These findings suggest that diabetes and excess body weight negatively influences bladder cancer prognosis and outcome. The increased risk of mortality due to diabetes was similar to that in the general population. Since retrospective studies are potentially susceptible to bias, future prospective studies on this subject are required.

Keywords: urinary bladder cancer, diabetes, overweight, obesity, all-cause mortality, cancer specific mortality, disease progression, disease recurrence

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INTRODUCTION

Urinary bladder cancer is quite prevalent, particularly in high-income settings (1). Bladder cancer is categorized as muscle invasive and non-muscle invasive: muscle invasive bladder cancers have low 5-year survival rates of ~35-40%, while non-muscle invasive bladder cancers have much higher survival rates (89-98%) accompanied by high five-year progression (~5-20%) and recurrence rates (~28-50%) (2–5). The resection of recurring tumors and subsequent treatment is often required, and expenditures associated with bladder cancer are often elevated compared to other cancers (6, 7). As such, frequent follow-up is advised for all bladder cancer patients post-treatment.

Efforts have been made to predict prognosis in bladder cancer patients using scoring tables (8). These tables focus on primary tumor characteristics. However, lifestyle factors such as obesity, smoking, as well as the presence of diabetes mellitus, can affect prognosis and modify follow-up schedules. Both diabetes mellitus and obesity are increasing in prevalence globally (9, 10), and insulin resistance and hyperinsulinemia have been proposed to affect bladder cancer risk and prognosis (11–13). Although several studies have looked at the link between body mass index and diabetes with cancer risk, few have looked at the impact of these factors on overall survival, tumor recurrence, and progression. Therefore, this current study aimed to pool available information and assess the impact of diabetes and elevated BMI on bladder cancer outcomes through a meta-analysis.

MATERIALS AND METHODS

Search Strategy

The literature search was designed and conducted based on PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines. We screened PubMed, Scopus, and Google Scholar academic databases for all English-language publications published prior to March 31, 2021. The search strategy incorporated medical topic heading (MeSH) terminology and free text words (**Supplementary Table 1**). The search aimed to identify studies reporting on the association of bladder cancer mortality, progression, and recurrence with diabetes and/or high body mass index. The primary outcomes for this meta-analysis were mortality (both all-cause and cancer specific), risk of cancer progression, and recurrence.

Selection Criteria and Methods

Study titles and abstracts were initially reviewed by two subject experts. Following this, the full texts for candidate studies were subsequently reviewed. Disagreements were resolved through discussion. Only studies that met all inclusion criteria were included for meta-analysis. Reference lists from included studies were manually screened for additional candidate studies.

Inclusion Criteria: To be included, studies must have been either retrospective record-based studies or prospective and have examined the impact of diabetes and/or high body mass index on bladder cancer outcomes.

Exclusion Criteria: Case reports, review articles, and other such studies were excluded. Studies that did not provide data on the outcomes of interest or did not examine the exposures of interest (diabetes or body mass index) were excluded.

Data Extraction and Quality Assessment

Relevant data from included studies was extracted using a set form by two independent reviewers. Extracted data included identification details, study setting, study design, sample size, follow-up duration, and main findings. Study quality was assessed using the Newcastle-Ottawa Quality Assessment Scale (14).

Statistical Analysis

This meta-analysis was conducted using STATA software (version 16.0) and reported effect sizes as pooled hazard ratios with 95% confidence intervals (CIs). Separate analyses were performed for diabetes and body mass index. Subgroup analysis was performed for tumor type (muscle invasive or non-muscle invasive), tumor stage, and tumor grade. I^2 was used to denote heterogeneity. We used random effects model as the included studies were diverse in their characteristics i.e., study subjects, geography, ethnicity, tumor characteristics etc. (15). P values under 0.05 was taken as statistically significant. Egger's test was employed to assess for publication bias.

RESULTS

Selection of Articles, Study Characteristics and Quality of Included Studies

Literature search revealed 789 candidate studies (**Figure 1**). Title screening removed 664 studies, and 91 were removed after abstract screening. The remaining 34 studies were then reviewed in detail, ultimately leaving 25 studies for inclusion in the meta-analysis. Nine of these studies focused on diabetes mellitus while 16 investigated body mass index (16–40) (**Supplementary Tables 2**, **3**). Of the nine studies that documented the link between diabetes and bladder cancer outcomes, three were conducted in South Korea, two in Taiwan, and one each in the Netherlands and USA. The remaining two studies were multicentered. Eight of the nine studies were retrospective analyses of patient data. Of the sixteen studies that investigated the link between body mass index and bladder cancer patient outcomes, three were performed in the USA, two in China, and one each in Germany, France, Turkey, Canada, and South Korea. The remaining six studies were multicentered. All sixteen studies were retrospective analyses of patient data. Study quality was good overall (**Supplementary Table 4**), with the majority of studies reporting appropriate processes for participant selection, outcome ascertainment, and controls.

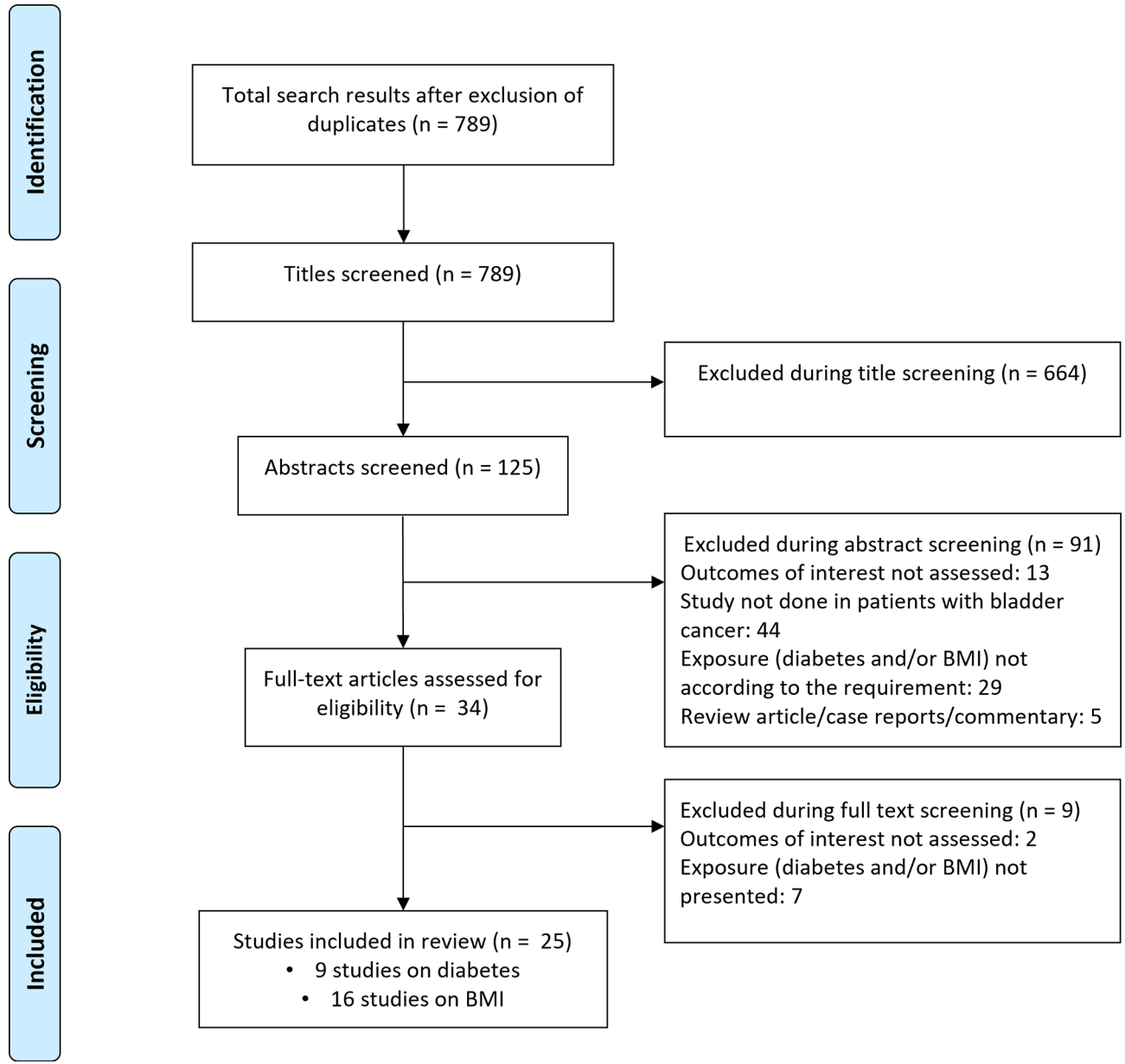

FIGURE 1 | Study inclusion process.

Association Between Diabetes and Bladder Cancer Outcome

Diabetic patients had significantly higher risks for both all-cause mortality (HR 1.24, 95% CI: 1.07, 1.44, n=3) and cancer-specific mortality (HR 1.67, 95% CI: 1.29, 2.16, n=7) than non-diabetics (**Figure 2**). Diabetic patients also had higher risks of disease progression (HR 1.54, 95% CI: 1.15, 2.06, n=8) and recurrence (HR 1.40, 95% CI: 1.32, 1.48, n=8) compared to non-diabetics (**Figure 2**). For disease progression as an outcome, we ran the analysis after excluding the study by Hwang EC et al. (24) and found the association to be still significant but comparatively lower in magnitude (HR 1.40, 95% CI: 1.17, 1.67, n=7). Egger's test did not show any evidence of publication bias for any of the examined outcomes (P=0.65 for all-cause mortality; P=0.43 for cancer specific mortality, P=0.72 for progression, and P=0.17 for recurrence).

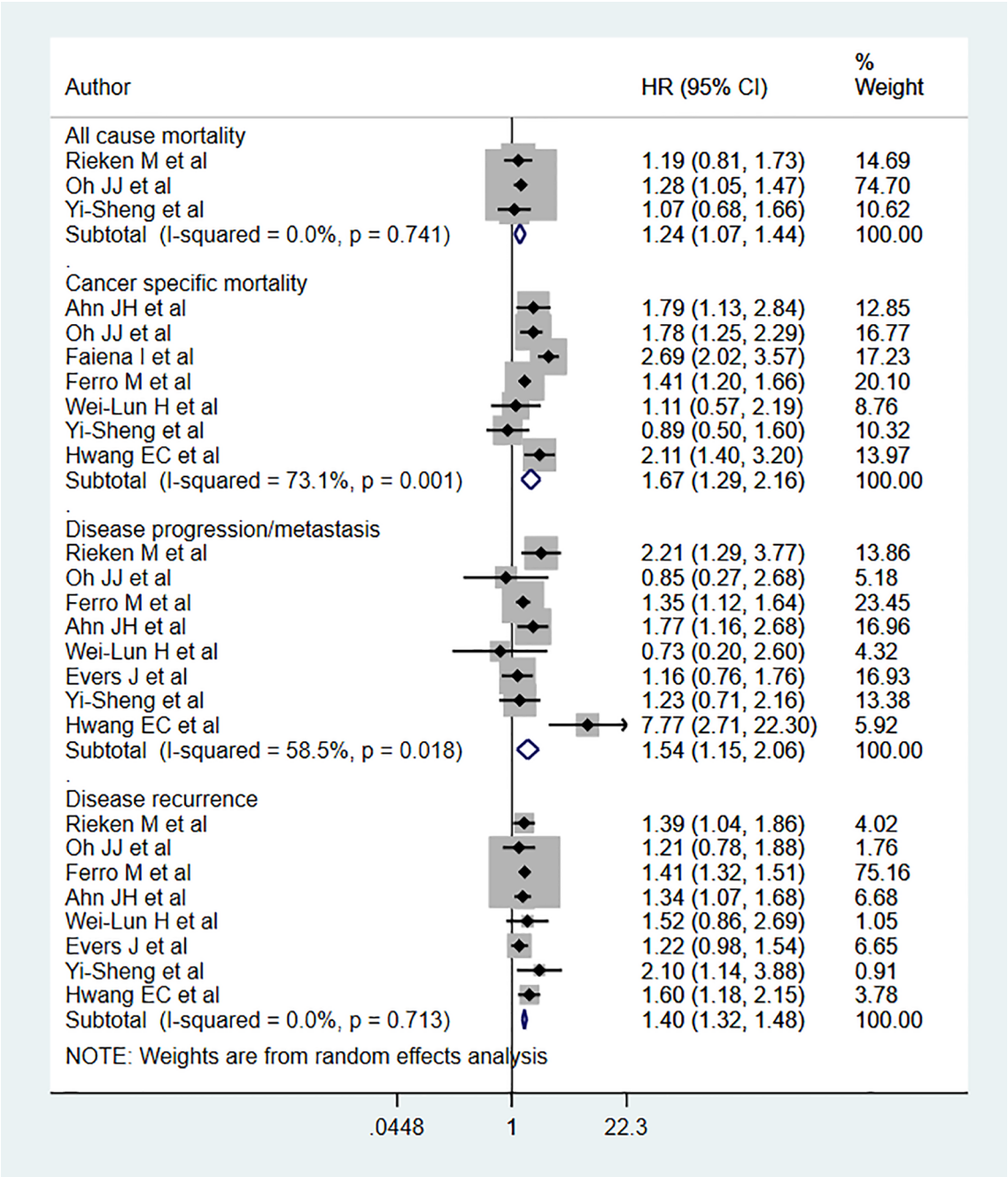

FIGURE 2 | Relationship between diabetes and bladder cancer outcomes.

Subgroup analysis showed that all-cause mortality risk among diabetics with an advanced tumor stage (≥T2) (HR 1.25, 95% CI: 1.07, 1.47, n=2) was elevated (**Table 1**). Cancer-specific mortality risk was high among diabetics with both early (PTa or T1) (HR 1.43, 95% CI: 1.23, 1.66, N=3] and advanced-stage tumors (HR 1.58, 95% CI: 1.04, 2.39, n=3). Disease progression risk was high in diabetics with early-stage tumors (HR 1.45, 95% CI: 1.16, 1.82, n=5), while recurrence risk was elevated in diabetics with both early (HR 1.39, 95% CI: 1.31, 1.48, N=5) and advanced-stage tumors (HR 1.54, 95% CI: 1.20, 1.97, n=3) (**Table 1**).

The risks of cancer-specific mortality (Low grade: HR 1.96, 95% CI: 1.44, 2.67, n=2; High grade: HR 1.39, 95% CI: 1.11, 1.76, n=4), disease progression (Low grade: HR 2.13, 95% CI: 1.00, 4.53, N=3; High grade: HR 1.39, 95% CI: 1.09, 1.76, N=5], and disease recurrence (Low grade: HR 1.34, 95% CI: 1.17, 1.55, n=3; High grade: HR 1.41, 95% CI: 1.32, 1.51, n=5) were elevated among diabetics with either low or high grade tumors. For all-cause mortality, risk was elevated only among diabetics with high grade tumors (HR 1.24, 95% CI: 1.07, 1.44, n=3) (**Table 1**).

Association Between Body Mass Index (BMI) and Bladder Cancer Outcome

No statistically significant association was noted between BMIs classified as overweight and all-cause mortality (HR 1.05, 95% CI: 0.74, 1.49, n=4) relative to the relationship between normal BMIs and all-cause mortality. Likewise, no association was noted for cancer specific mortality (HR 0.92, 95% CI: 0.75, 1.13, n=6), disease progression (HR 1.45, 95% CI: 0.79, 2.66, n=3), or recurrence (HR 1.22, 95% CI: 0.80, 1.87, n=7) (**Figure 3**). Egger's test revealed no evidence of publication bias for any of the considered outcomes (P=0.33 for all-cause mortality; P=0.24 for cancer specific mortality, P=0.57 for progression, and P=0.37 for recurrence)

Subgroup analysis showed a decrease risk of cancer-specific mortality for overweight individuals with muscle-invasive bladder cancer (MIBC) (HR 0.77, 95% CI: 0.67, 0.89, n=3) compared to normal BMI counterparts (**Table 2**). All-cause mortality was also decreased for overweight patients with low grade tumors (HR 0.80, 95% CI: 0.69, 0.93, n=1). However, the risk of recurrence increased (HR 1.42, 95% CI: 1.11, 1.81, n=2). No significant differences were noted in any other subgroup analyses (**Table 2**).

Compared to patients with normal BMI, obese patients had elevated risk for disease progression (HR 1.88, 95% CI: 1.41, 2.50, n=3) and recurrence (HR 1.60, 95% CI: 1.06, 2.40, n=7). However, no statistically significant risk change was noted for all-cause mortality (HR 1.33, 95% CI: 0.85, 2.07, n=3) or cancer-specific mortality (HR 0.94, 95% CI: 0.54, 1.66, = 5) (**Figure 4**). Subgroup analysis showed that obese patients with muscle-invasive bladder cancer (MIBC) had elevated risk for all-cause mortality (HR 1.57, 95% CI: 1.04, 2.36, n=2) (**Table 3**). Moreover, obese patients with non-muscle invasive bladder cancer showed elevated risk for cancer specific mortality (HR 1.51, 95% CI: 1.05, 2.16, n=2), disease progression (HR 1.88, 95% CI: 1.41, 2.50, n=3), and recurrence (HR 2.01, 95% CI: 1.39, 2.90, n=5) (**Table 3**). Obese patients also presented elevated risk of progression and recurrence regardless of whether the patient had low- or high-grade cancer. Obese patients with advanced-stage tumors (≥T2) showed higher risk for all-cause mortality (HR 1.57, 95% CI: 1.04, 2.36, n=2) and disease recurrence (HR 1.66, 95% CI: 1.46, 1.89, n=2) (**Table 3**).

DISCUSSION

The current meta-analysis aimed to examine the relationship between bladder cancer outcomes and diabetes or body weight. This study found that diabetic patients had significantly elevated risk for all-cause mortality, cancer specific mortality, disease progression, and recurrence. Obese patients also showed significantly elevated risk for disease progression and recurrence. However, no change in risk for all-cause mortality and cancer specific mortality was noted.

The deleterious influence of diabetes on cancer-related outcomes has been previously documented (41–43). However, the exact underlying mechanisms for how diabetes and elevated BMIs can affect cancer-related outcomes are unclear. Researchers have hypothesized that hyperinsulinemia or hyperglycemia are

TABLE 1 | Subgroup analysis for diabetes as a bladder cancer risk factor.

	Pooled effect size (Hazard ratio; HR) (95% Confidence Interval)			
	Stage of tumor		Grade of tumor	
	Early stage (PTa or T1)	Advanced (≥T2)	Low	High
All-cause mortality	N=1 1.19 (0.81, 1.74)	N=2 1.25 (1.07, 1.47)	—	N=3 1.24 (1.07, 1.44)
Cancer specific mortality	N=3 1.43 (1.23, 1.66)	N=3 1.58 (1.04, 2.39)	N=2 1.96 (1.44, 2.67)	N=4 1.39 (1.11, 1.76)
Risk of progression	N=5 1.45 (1.16, 1.82)	N=3 1.97 (0.59, 6.58)	N=3 2.13 (1.00, 4.53)	N=5 1.39 (1.09, 1.76)
Risk of recurrence	N=5 1.39 (1.31, 1.48)	N=3 1.54 (1.20, 1.97)	N=3 1.34 (1.17, 1.55)	N=5 1.41 (1.32, 1.51)

Out of the 9 studies included in the meta-analysis, only one study had subjects with muscle invasive bladder. Similarly, in only one study, majority of the subjects had tumor size>3 cm; In all the studies, patients did not have >2 tumors and none of the studies reported presence of carcinoma in situ in majority of the subjects. The modality of treatment in almost all the studies was transurethral resection of bladder with/without adjuvant therapy. Therefore, due to lack of variation for these variables among the included studies, sub-group analysis was not conducted on these variables.

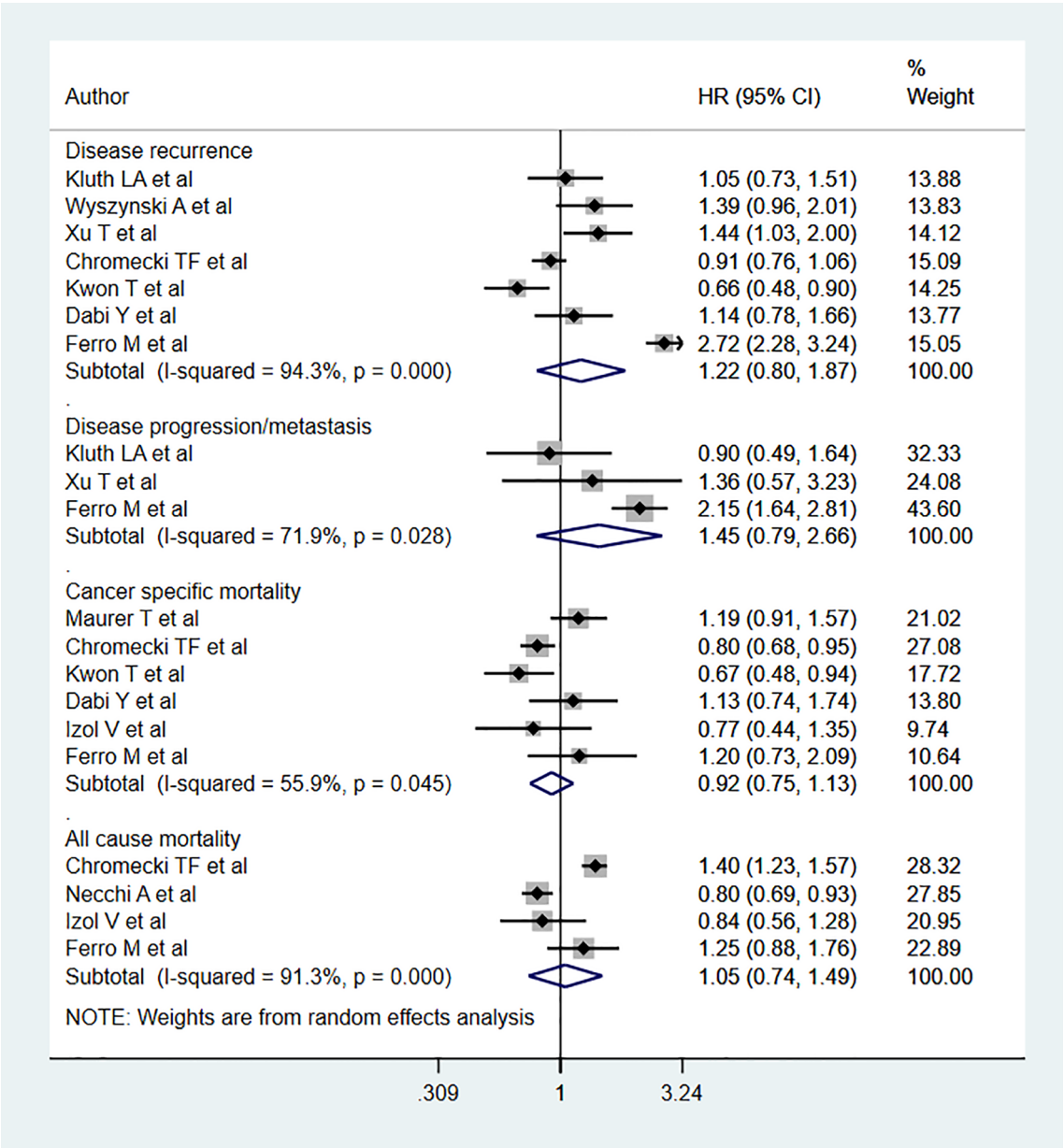

FIGURE 3 | Bladder cancer patient outcomes in overweight and normal BMI patients.

involved, as chronic hyperinsulinemia or hyperglycemia has been shown to promote tumor cell proliferation and metastasis (44–47). Similarly, elevated levels of insulin-like growth factor (IGF)-1 induces cellular proliferation while inhibiting apoptosis (44–47). Excess adiposity also creates a pro-inflammatory environment, and this may contribute to poorer prognostic outcomes in cancers. Diabetes has also been linked to increased risk for urinary tract infection (UTI), which in turn has been linked to elevated risk for bladder cancer onset, recurrence, and progression (48, 49).

While the findings of the meta-analysis indicate that diabetes is associated with mortality, recurrence and tumor progression, there are many considerations to take into account. First and foremost, we did not explore whether there was a difference between the non-bladder cancer death risk and the bladder cancer death risk in diabetic patients as none of the included studies had non-bladder cancer subjects. Having this analysis would have been important to understand whether presence of diabetes increased the risk of mortality in subjects with bladder cancer, over and above the risk of mortality in the general

TABLE 2 | Subgroup analysis for bladder cancer outcomes in overweight patients relative to normal BMI.

	Type of tumor		Grade of tumor		Stage of tumor	
	Non-muscle invasive (NMIBC)	Muscle invasive (MIBC)	Low	High	Early stage (PTa or T1)	Advanced (≥T2)
All-cause mortality	N=2 0.97 (0.63, 1.50)	N=2 1.13 (0.69, 1.85)	N=1 0.80 (0.69, 0.93)	N=3 1.20 (0.91, 1.58)	N=2 0.97 (0.63, 1.50)	N=2 1.13 (0.69, 1.85)
Cancer specific mortality	N=3 1.18 (0.95, 1.45)	N=3 0.77 (0.67, 0.89)	N=1 1.19 (0.91, 1.56)	N=5 0.84 (0.70, 1.02)	N=2 0.86 (0.49, 1.52)	N=4 0.96 (0.75, 1.22)
Risk of progression	N=3 1.45 (0.79, 2.66)	—	N=1 1.36 (0.57, 3.24)	N=2 1.45 (0.62, 3.40)	N=3 1.45 (0.79, 2.66)	—
Risk of recurrence	N=5 1.48 (0.96, 2.26)	N=2 0.80 (0.59, 1.09)	N=2 1.42 (1.11, 1.81)	N=5 1.15 (0.65, 2.03)	N=5 1.31 (0.75, 2.29)	N=2 0.95 (0.80, 1.14)

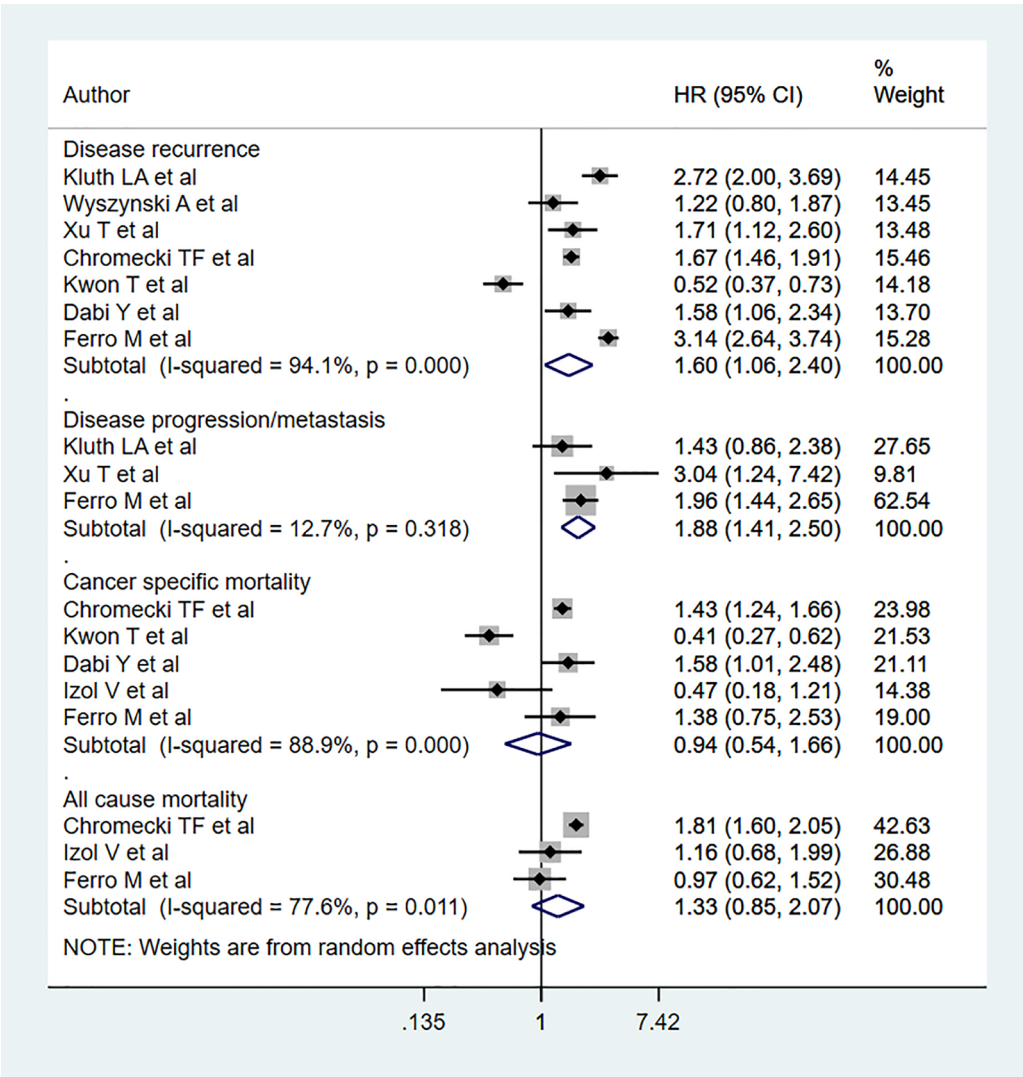

FIGURE 4 | Bladder cancer patient outcomes in obese and normal BMI patients.

TABLE 3 | Subgroup analysis for bladder cancer outcomes in obese patients relative to normal BMI.

	Effect size (Hazard ratio; HR) (95% Confidence Interval)					
	Type of tumor		Grade of tumor		Stage of tumor	
	Non-muscle invasive (NMIBC)	Muscle invasive (MIBC)	Low	High	Early stage (PTa or T1)	Advanced (≥T2)
All-cause mortality	N=1 0.97 (0.62, 1.52)	N=2 1.57 (1.04, 2.36)	—	N=3 1.33 (0.85, 2.07)	N=1 0.97 (0.62, 1.52)	N=2 1.57 (1.04, 2.36)
Cancer specific mortality	N=2 1.51 (1.05, 2.16)	N=3 0.68 (0.25, 1.84)	—	N=5 0.94 (0.54, 1.66)	N=2 0.74 (0.22, 2.42)	N=3 1.26 (0.83, 1.91)
Risk of progression	N=3 1.88 (1.41, 2.50)	—	N=1 3.04 (1.24, 7.44)	N=2 1.79 (1.36, 2.37)	N=3 1.88 (1.41, 2.50)	—
Risk of recurrence	N=5 2.01 (1.39, 2.90)	N=2 0.94 (0.30, 2.96)	N=2 1.45 (1.04, 2.01)	N=5 1.65 (1.01, 2.76)	N=5 1.57 (0.80, 3.08)	N=2 1.66 (1.46, 1.89)

population or subjects with no bladder cancer. Available evidence indicates that the risk of mortality due to diabetes in the general population is similar to the estimates from the current meta-analysis involving patients with bladder cancer. This may imply that presence of diabetes among subjects with bladder cancer does not significantly increase the risk of mortality, when compared to the general population. However, this finding should not be interpreted as lack of benefit in terms of survival among bladder cancer patients through efforts aimed at better glycemic control. Diabetes is a multifactorial disease where duration, glycosylated hemoglobin levels (HbA1c), glycemic variability, age of patients and sex constitute a cluster with very different impact on clinical peculiarity. In the included studies, majority of the participants had type 2 diabetes and were on oral hypoglycemics. The participants were usually aged more than 60 years of age and majority were males. A growing amount of evidence supports a link between obesity-associated inflammation and cancer incidence and progression (50). Obesity leads to a stage of chronic inflammation with upsurge in inflammatory cytokines leading to an increase the number of cells with tumor-forming capabilities (51). Inflammatory markers such as IL-6, TNF-α, and prostaglandin E2 are all elevated in obese patients. Another important issue is that the levels of leptin are usually higher in patients with obesity. It is well established that leptin induces the expression of pro-inflammatory and pro-tumor cytokines including IL-1, IL-6 and TNF-α (52). The pro-tumor role of leptin is usually due to its role in the promotion of angiogenesis and in enhancing the proliferation and survival of tumor cells. On the other hand, it also inhibits apoptosis, thereby, leading to progression and metastasis (53).

This study highlights the need for close monitoring, supervision and follow up in urinary bladder cancer patients presenting with either elevated BMI and/or diabetes in order to alleviate the risk of mortality, recurrence and disease progression. This study did have several limitations. First, almost all included studies were retrospective, making it difficult to account for any adjustment for potential confounding factors. There is a need for future prospective studies on this issue in order to provide reliable and unbiased evidence. One of the obvious limitations of this meta-analysis is the lack of evidence synthesis on the association of glycemic control (using HbA1c) with the outcomes. This could not be done because of included studies not reporting this association. Further, this was not the primary analysis planned and future research should aim to explore this association. Another limitation relates to the inclusion of multicentric studies and the lack of information concerning protocol harmonization across centres. The study attempted to derive an association of diabetes and BMI with mortality, progression or cancer recurrence. However, it should be noted that a significant overlap between diabetes, obesity, insulin and hypoglycemic agents on cancer outcome could be a major cause of bias in this study. We did not find a statistically significant association between BMI classified as overweight and all-cause mortality. However, there is a limitation to it. There is no unique reference range/operational definition for BMI that was being used to categorize overweight in the included studies. Further, there is a difference in the reference range based on the gender of the participants. While it would have been useful to perform an adjunctive analysis according to sex and related BMI, such an analysis could not be done because of lack of reported gender specific findings in the studies included in the meta-analysis. Finally, retrospective studies largely assessed the presence or absence of diabetes based on medical records/ treatment history, and this could result in bias concerning classification. Similarly, different studies used different cut-offs to define "overweight" and "obese". These discrepancies may lead to inter-study heterogeneity.

CONCLUSION

The current meta-analysis suggests that both diabetes and excessive BMI can potentially negatively influence bladder cancer outcomes such as mortality, progression, and recurrence. The risk of mortality due to diabetes in patients

with bladder cancer was similar to that in the general population. However, this finding does not undermine the need for better glycemic control in these patients in order to improve survival. Given that retrospective study designs may be subject to certain biases, there is a need for prospective studies investigating this relationship.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

YL conceived and designed the study. JT and YL did literature search, analysis and wrote the paper. YL reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.699732/full#supplementary-material

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Greater Body Fatness Is Associated With Higher Protein Expression of LEPR in Breast Tumor Tissues: A Cross-Sectional Analysis in the Women's Circle of Health Study

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Background: The mechanisms underlying the association of overall and central body fatness with poorer breast cancer outcomes remain unclear; altered gene and/or protein expression of the adipokines and their receptors in breast tumors might play a role.

Methods: In a sample of Black and White women with primary invasive breast cancer, we investigated associations of body mass index (BMI), waist circumference, hip circumference, waist-to-hip ratio (WHR), fat mass index (FMI), and percent body fat with protein expression (log-transformed, n = 722) and gene expression (log2-transformed, n = 148) of leptin (LEP), leptin receptor (LEPR), adiponectin (ADIPOQ), and adiponectin receptors 1 and 2 (ADIPOR1, ADIPOR2). Multivariable linear models, adjusting for race, menopausal status, and estrogen receptor status, were used to assess these associations, with Bonferroni correction for multiple comparisons.

Results: In multivariable models, we found that increasing BMI (β = 0.0529, 95% CI: 0.0151, 0.0906) and FMI (β = 0.0832, 95% CI: 0.0268, 0.1397) were associated with higher *LEP* gene expression, corresponding to 34.5% and 38.3% increases in *LEP* gene expression for a standard deviation (SD) increase in BMI and FMI, respectively. Increasing BMI (β = 0.0028, 95% CI: 0.0011, 0.0045), waist circumference (β = 0.0013, 95% CI: 0.0005, 0.0022), hip circumference (β = 0.0015, 95% CI: 0.0007, 0.0024), and FMI (β =

0.0041, 95% CI: 0.0015, 0.0067) were associated with higher LEPR protein expression. These associations equate to 16.8%, 17.6%, 17.7%, 17.2% increases in LEPR protein expression for a 1-SD increase in BMI, waist circumference, hip circumference, and FMI, respectively. Further, these associations were stronger among White and postmenopausal women and ER+ cases; formal tests of interaction yielded evidence of effect modification by race. No associations of body fatness with LEP protein expression, *LEPR* gene expression, or protein or gene expression of ADIPOQ, ADIPOR1, and ADIPOR2 were found.

Conclusions: These findings support an association of increased body fatness – beyond overall body size measured using BMI – with higher *LEP* gene expression and higher LEPR protein expression in breast tumor tissues. Clarifying the impact of adiposity-related adipokine and adipokine receptor expression in breast tumors on long-term breast cancer outcomes is a critical next step.

Keywords: adiposity, breast cancer, leptin (LEP), leptin receptor (LEPR), adiponectin (ADIPOQ), adiponectin receptors 1 and 2 (ADIPOR1, ADIPOR2), protein expression, gene expression

INTRODUCTION

Epidemiologic evidence suggests that increasing obesity, measured using body mass index (BMI), is associated with elevated risk of postmenopausal breast cancer (1, 2) and poorer breast cancer outcomes among both pre- and postmenopausal women (2–4). However, differences have been observed by estrogen receptor (ER) status (3, 5). While increased premenopausal obesity is associated with increased risk of ER- but not ER+ disease, postmenopausal obesity is similarly associated with increased risk of both ER- and ER+ disease (5, 6). On the other hand, increasing waist-to-hip ratio (WHR) is associated with increased risk of ER+ disease among premenopausal women and with increased risk of both ER+ and ER- disease among postmenopausal women (7).

While the molecular mechanisms underlying the impact of overall and central obesity on poorer breast cancer outcomes are not well understood, it has been hypothesized that the biological effects of the adipokines, adiponectin (ADIPOQ) and leptin (LEP), which are secreted by adipocytes (8–13), and their respective receptors (adiponectin receptors 1 and 2 [ADIPOR1, ADIPOR2] and leptin receptor [LEPR], respectively) might play a role. Further, exploration of the relationship between central adiposity (rather than overall body size as measured by BMI) and adipokines and adipokine receptors might be the missing link. Circulating ADIPOQ levels decrease with increasing BMI (14–16) and are associated with increased breast cancer risk (17–20). Conversely, circulating LEP levels increase with increasing BMI (21, 22) and are associated with increased breast cancer risk in some studies (17, 23–25). Less is known about adipokine receptor protein and gene expression levels in breast tumor tissues or their associations with more accurate and specific measures of body fatness derived from anthropometry (e.g., waist circumference, hip circumference, waist-to-hip ratio [WHR]) or from bioelectrical impedance analysis (BIA) (e.g., fat mass index [FMI], percent body fat [BF%]). These data might provide novel insights about the impact of body fatness and adiposity-related biomarker expression (at the tumor level) on breast cancer outcomes.

ADIPOQ is the most abundantly secreted adipokine by adipocytes (15, 26), and along with its receptors, is expressed in histologically normal and malignant breast tissues (27, 28). ADIPOQ has anti-inflammatory and anti-atherogenic properties (26, 29), inhibits cellular proliferation, and promotes apoptosis (10, 13, 30), implying a protective role in breast carcinogenesis. LEP, also secreted by adipocytes, is expressed in histologically normal and malignant breast cells, as is the LEPR (31, 32), LEP, once bound to LEPR, induces the activation of several signaling pathways, promotes cell growth and proliferation, and promotes angiogenesis (33–38).

Data from our prior research were the first to examine correlations between circulating ADIPOQ and LEP levels in plasma and levels within the breast, demonstrating that circulating adipokine levels are generally poor surrogates for levels within the local organ (39). More recently, we demonstrated that adipokine and adipokine receptor protein and gene expression in breast tumor tissues are associated with more aggressive tumor features associated with worse prognosis (40, 41). Specifically, lower LEPR protein expression was associated with ER- status, triple-negative (TN) subtype (40), while lower gene expression of *ADIPOQ*, *ADIPOR2*, *LEP*, and *LEPR* were associated with more aggressive breast tumor features, including higher tumor grade, larger tumor size, ER- status, and human epidermal growth factor receptor 2 (HER2)-enriched and TN subtypes (41).

Abbreviations: ADIPOQ, adiponectin; ADIPOR1, adiponectin receptor 1; ADIPOR2, adiponectin receptor 2; BIA, bioelectrical impedance analysis; BMI, body mass index; CI, confidence interval; CT, computed tomography; DCIS, ductal carcinoma in situ; ER, estrogen receptor; ESA, effective staining area; ESI, effective staining intensity; FFPE, formalin-fixed paraffin-embedded; H&E, hematoxylin and eosin; HER2, human epidermal growth factor receptor 2; LEP, leptin; LEPR, leptin receptor; SD, standard deviation; TMA, tissue microarray; TN, triple negative; WCHS, Women's Circle of Health Study; WHR, waist-to-hip ratio.

In the current study, we hypothesize that measures of body fatness are associated with *LEPR*, *ADIPOR1*, and *ADIPOR2* expression profiles in the breast tumor microenvironment, which might contribute mechanistically to the development of more aggressive breast tumor phenotypes and poorer prognosis. To test this, we investigated associations of general obesity (BMI), body fat distribution (waist circumference, hip circumference, WHR), and body composition (FMI, BF%) with protein and gene expression of the adipokines and adipokine receptors in breast tissue specimens from participants in the Women's Circle of Health Study (WCHS).

MATERIALS AND METHODS

Study Sample and Data Collection

Study participants were women diagnosed with primary invasive breast cancer from 2001 through 2015 and enrolled in the WCHS (40, 41). Briefly, WCHS enrolled newly diagnosed breast cancer cases with histologically confirmed ductal carcinoma *in situ* (DCIS, stage 0) or invasive breast cancer (stages I–IV), who self-identified as either Black/African American or White, were 20–75 years of age, able to complete an interview in English, and had no history of cancer except non-melanomatous skin cancer. Data collection for the WCHS was conducted through in-person assessments (approximately 10 months after diagnosis) and included computer-assisted interviewer-administered questionnaires, as well as standardized protocols for taking anthropometric measurements during a home visit, including height, weight, waist circumference, and hip circumference, and body composition using a portable BIA scale (42). The baseline interview ascertained information on sociodemographic factors as well as established or probable breast cancer risk factors, including family and personal health history, reproductive history, hormone therapy use, and lifestyle exposures.

Nearly all WCHS participants (98%) consented to medical records release and for these participants, medical and pathology records were requested and retrieved from providers and institutions where participants reported receiving breast cancer care. Relevant clinical and breast tumor clinicopathologic data were abstracted and entered in an electronic database (43, 44).

Collection of Archived Breast Tumor Specimens and Tissue Microarray Construction

Tumor blocks and/or slides for WCHS participants were retrieved from hospitals upon written consent, with a retrieval rate of approximately 85%. Upon receipt at the Data Bank and Biorepository (DBBR) at Roswell Park Comprehensive Cancer Center, a board-certified pathologist (TK) reviewed hematoxylin and eosin (H&E) slides and circled areas where cores were selected for tissue microarray (TMA) construction. TMA cores ranged in size from 0.6 mm to 1.2 mm in diameter, and most WCHS participants' tumors were represented by at least two TMA cores (range: 1 to 6 cores), which were placed into a recipient formalin-fixed paraffin-embedded (FFPE) block. The location of each core was recorded in a detailed TMA map file. The completed TMAs were stored at room temperature.

Protein Expression Analysis

For each WCHS participant included in the protein expression analysis (n = 722), immunohistochemistry (IHC) was used to stain TMAs of breast tumor specimens for LEP, LEPR, ADIPOQ, ADIPOR1, and ADIPOR2 as previously described (40). Briefly, IHC staining was performed using Ventana Discovery XT Automated Slide Stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). Deparaffinization, antigen retrieval, blocking, DAB detection, counterstain, post-counterstain, and slide cleaning steps were automated on the Discovery XT. Primary antibodies and secondary antibodies were manually applied at programmed steps. The following primary antibodies were used: rabbit monoclonal OB (LEP) antibody (1:40 dilution; Santa Cruz, cat #sc-842), mouse monoclonal Ob-R (LEPR) antibody (1:25 dilution; Santa Cruz, cat #sc-8391), mouse monoclonal adiponectin antibody (1:30 dilution; Abcam, cat #ab22554), rabbit monoclonal ADIPOR1 antibody (1:350 dilution; Abcam, cat #ab126611), and goat polyclonal ADIPOR2 antibody (1:25 dilution; Abcam, cat #ab77612). Optimal staining on control slides (human breast tissue TMAs) was obtained for each individual antibody. IHC was performed using the optimized conditions on the experimental WCHS TMA slides as well as on additional control slides. Primary antibodies were incubated at 37°C for 1–2 h; secondary antibodies were incubated at 37°C for 1 h, followed by either the DAB Map Detection Kit (Ventana, 760-124) or ChromoMap DAB kit (Ventana, 760-159). Slides were counterstained with hematoxylin (Ventana, 760-2021)and bluing reagent (Ventana, 760-2037) before cover slipping. A digital pathology analysis platform (VisioPharm, Hoersholm Denmark) was used to quantify protein expression of the adipokine receptors on each tissue core (45). Quantitative results were reported as a protein expression score defined as effective staining intensity (ESI) within the effective staining area (ESA) (45). Specimen artifacts, such as tissue folding were manually excluded from quantification. A board-certified pathologist (MAC) semi-quantitatively evaluated IHC expression for each tissue core stained (45). Semi-quantitative expression results were reported as: 0 (negative), 1 (weak expression), 2 (moderate expression), or 3 (strong expression). We observed high concordance between unsupervised, quantitative scores and pathologist-generated, semi-quantitative scores for LEP ($r = 0.70$, $P<0.0001$) and LEPR ($r = 0.71$, $P<0.0001$) (40). In the present analysis we included only quantitative protein expression data for LEP, LEPR, ADIPOQ, ADIPOR1, and ADIPOR2, which were averaged for participants with multiple TMA cores. Log-transformed protein expression data were used in the subsequent analysis.

Gene Expression Analysis

For each WCHS participant included in the gene expression analysis (n = 148), RNA was extracted from two 10μm curls (from representative breast tumor blocks without any pre-selection based on either the tumor or stromal contents so as

to maintain and capture the entire tumor lesion and surrounding microenvironment) using the High Pure FFPET RNA Isolation Kit (Roche Molecular Systems, Inc., Pleasanton, CA, USA) and quantified using Qubit and Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Gene expression of *ADIPOQ, ADIPOR1*, *ADIPOR2*, LEP, and *LEPR* were quantitated using NanoString nCounter® technology (NanoString Technologies, Seattle, WA, USA) (41). Raw count data were subjected to a series of normalization steps, including positive controls, housekeeping genes, and background subtraction, and the normalized data were log2-transformed and used in subsequent analyses (41).

Statistical Analyses

Descriptive statistics (mean and standard deviation [SD] and frequency and proportions) were used to describe the study sample and Pearson's correlation analysis was used to assess pairwise correlations between adipokine receptor protein and gene expression. Multivariable linear regression models were utilized to evaluate the associations of BMI, waist circumference, hip circumference, WHR, FMI, and BF% with protein and gene expression of the adipokines and adipokine receptors. The difference in protein and gene expression per increase in SD of body fatness measures was also estimated, and a percentage increase was estimated as [exp(beta) - 1] x 100%. Models were adjusted for age, race, menopausal status, and ER status. All reported *P*-values are two-sided and $P<0.05$ was considered statistically significant. To account for multiple comparisons, we used Bonferroni correction with a criterion of $P<0.0083$ (i.e., 0.05/6) for statistical significance, given that there were six tests of association for protein and gene expression of each marker of interest. Analyses were performed using STATA (version 17, StataCorp, College Station, TX).

RESULTS

Sociodemographic and tumor characteristics of the study sample included in the protein expression and the gene expression analytic samples are shown in **Table 1**. Across both groups, most participants met the criteria for increased metabolic risk (46, 47) based on overall obesity (BMI >30 kg/m^2 [50.6%]), central obesity (waist circumference >88 cm [74.4%] and/or WHR >0.85 [64.1%]), elevated/abnormal FMI (≥9.5 kg/m^2 [73.4%]), and BF% (>35% [76.2%]).

There was no significant correlation between LEP protein and *LEP* gene expression (r = 0.11, P = 0.21), a weak positive correlation between LEPR protein and *LEPR* gene expression (r = 0.29, P = 0.0006), a weak positive correlation between ADIPOQ protein and *ADIPOQ* gene expression (r = 0.18, P = 0.04), no significant correlation between ADIPOR1 protein and *ADIPOR1* gene expression (r = -0.03, P = 0.69), and a very weak positive correlation between ADIPOR2 protein and *ADIPOR2* gene expression (r = 0.18, P = 0.04). In models adjusting for age, race, menopausal status, and ER status, we found that there were no significant associations between measures of body fatness and LEP protein expression (**Table 2**). In contrast, we found that women with increasing BMI (β = 0.0529, 95% CI: 0.0151, 0.0906), waist circumference (β = 0.0247, 95% CI: 0.0065, 0.0429), hip circumference (β = 0.0259, 95% CI: 0.0066, 0.0453), FMI (β = 0.0832, 95% CI: 0.0268, 0.1397), and BF% (β = 0.0396, 95% CI: 0.0025, 0.0767) had higher *LEP* gene expression. Only the findings for BMI and FMI were significant with correction for multiple comparisons and corresponds to 34.5% and 38.3% increases in *LEP* gene expression for a 1-SD increase in BMI and FMI, respectively. Women with greater body fatness had significantly higher LEPR protein expression: BMI (β = 0.0028, 95% CI: 0.0011, 0.0045), waist circumference (β = 0.0013, 95% CI: 0.0005, 0.0022), hip circumference (β = 0.0015, 95% CI: 0.0007, 0.0024), and FMI (β = 0.0041, 95% CI: 0.0015, 0.0067) (**Table 3**). These associations equate to 16.8%, 17.6%, 17.7%, 17.2% increases in LEPR protein expression for a 1-SD increase in BMI, waist circumference, hip circumference, and FMI, respectively. WHR was not associated with LEPR protein expression. Upon further adjustment for waist circumference, the observed associations between BMI (P = 0.08), hip circumference (P = 0.13), and BF% (P = 0.26) were consistent but attenuated, while the association for FMI was slightly stronger (β = 0.0055, 95% CI: 0.0005, 0.0010; 24.1% increase in LEPR protein expression), although not statistically significant (data not shown). Conversely, we found no association between body fatness and *LEPR* gene expression. Associations between body fatness and ADIPOQ expression (**Table 4**), ADIPOR1 expression (**Table 5**) and ADIPOR2 expression (**Table 6**) were not statistically significant, but the coefficients suggested that increasing body fatness might be associated with lower protein expression of ADIPOQ, ADIPOR1, and ADIPOR2, lower *ADIPOQ* gene expression. and higher gene expression of *ADIPOR1* and *ADIPOR2*.

Given the multivariable-adjusted associations observed between measures of body fatness and LEPR protein expression levels, we explored potential differences by race (**Table 7**), menopausal status (**Table 8**), and ER status (**Table 9**). Qualitatively, our observation that increasing body fatness measures are associated with higher LEPR protein expression appeared stronger among White women, postmenopausal women, and ER+ cases. Formal tests of interaction yielded statistically significant evidence of effect modification by race for some body fatness measures (BMI, P = 0.041; FMI, P = 0.016; and BF%, P = 0.019), but not others (waist circumference, P = 0.080; hip circumference, P = 0.086) (data not shown). However, we observed no evidence of effect modification by menopausal status (P-values for all body fatness measures >0.05), and limited evidence of effect modification by ER status (BMI, P = 0.318; waist circumference, P = 0.093; hip circumference, P = 0.059; WHR, P = 0.821; FMI, P = 0.250; and BF%, P = 0.553) (data not shown).

DISCUSSION

Building on our prior research, here we examined the associations of body fatness measures with protein and gene expression of the adipokines, LEP and ADIPOQ, and the adipokine receptors, LEPR, ADIPOR1, and ADIPOR2 in breast tumor tissues. To our

TABLE 1 | Select characteristics of analytic samples included in the adipokine receptor protein expression and gene expression analysis.

	Protein expression, N = 722[a]	Gene expression, N = 148[b]
Sociodemographic and clinical characteristics	**n (%)**	**n (%)**
Age at diagnosis (years), mean ± SD	52.58 ± 10.83	53.08 ± 10.34
Menopausal status		
Premenopausal	325 (46.43)	68 (47.22)
Postmenopausal	375 (53.57)	76 (52.78)
Race		
Black/African American	541 (77.29)	109 (75.69)
White	159 (22.71)	35 (24.31)
Body mass index (kg/m^2), mean ± SD	30.72 ± 6.99	30.89 ± 7.50
Waist circumference (cm), mean ± SD	98.63 ± 15.47	99.15 ± 15.66
Hip circumference (cm), mean ± SD	112.13 ± 13.30	111.86 ± 13.88
Waist-to-hip ratio, mean ± SD	0.87 ± 0.08	0.88 ± 0.07
Fat mass index, mean ± SD	12.44 ± 4.82	12.61 ± 5.27
Percent body fat (%),mean ± SD	39.30 ± 7.77	39.20 ± 8.07
Breast tumor characteristics		
Tumor grade		
Well differentiated	107 (16.85)	13 (9.03)
Moderately differentiated	219 (34.49)	45 (31.25)
Poorly differentiated	309 (48.66)	86 (59.72)
Tumor size		
<1.0 cm	149 (20.64)	22 (14.86)
1.0-2.0 cm	281 (38.92)	64 (43.24)
>2.0 cm	292 (40.44)	62 (41.89)
AJCC stage		
Stage 0	62 (8.96)	1 (0.72)
Stage I	257 (37.14)	54 (39.13)
Stage II	271 (39.16)	66 (47.83)
Stage III	96 (13.87)	14 (10.14)
Stage IV	6 (0.87)	3 (2.17)
ER status		
ER+	505 (70.14)	84 (56.76)
ER-	215 (29.86)	64 (43.24)
HER2 status		
HER2-	412 (81.58)	112 (75.68)
HER2+	93 (18.42)	36 (24.32)

[a]*In the protein expression sample, age was missing for 22 (3%); BMI was missing for 23 (3.2%); waist circumference, hip circumference, and waist-to-hip ratio were missing for 33 (4.6%); fat mass index was missing for 66 (9.1%); percent body fat was missing for 64 (8.9%); tumor grade was missing for 87 (12%); tumor stage was missing for 30 (4.2%); ER status was missing for 2 (0.3%), and HER2 status was missing for 18 (2.5%) participants.*

[b]*In the gene expression sample, age, menopausal status, race, and BMI was missing for 4 (2.7%) participants; waist circumference, hip circumference, and waist-to-hip ratio were missing for 5 (3.4%) participants; fat mass index and percent body fat were missing for 12 (8.1%) participants; tumor grade was missing for 4 (2.7%) participants; and tumor stage was missing for 10 (6.8%) participants.*

knowledge, this is the first study to investigate these associations in women with breast cancer. Partially consistent with our hypothesis, we found that greater body fatness is associated with increased *LEP* gene expression and LEPR protein expression, although we observed no associations between body fatness and *LEPR* gene expression, nor with protein or gene expression of ADIPOQ, ADIPOR1, and ADIPOR2.

Past studies show that BMI is positively associated with circulating leptin concentrations and inversely associated with circulating adipokine concentrations, which are associated with increased risk of some obesity-related cancers including breast cancer (reviewed by Yoon et al. (48)). Our finding that increasing measures of body fatness are positively associated with LEPR protein expression in breast tumors independent of age and menopausal status (with correction for multiplicity) support the hypothesis that LEPR protein expression in breast tumor tissues plays a role in breast carcinogenesis (49–54). Interestingly, our analysis showed significant effect modification by race (stronger among White women) and marginally significant effect modification by ER status (suggestion of stronger associations among ER+ cases, although our analysis was underpowered given the small sample of ER- cases). These findings further highlight the complex interplay among LEPR protein expression, adiposity, race, and breast tumor phenotype (55–58), which might require more precise adiposity measures, and identification and refinement of adiposity-associated biomarkers within breast tumor tissues that can predict breast cancer outcomes. We observed no significant associations between body fatness and *LEPR* gene expression, but we previously showed that gene expression of *LEPR* is significantly lower in ER- and TN breast tumors relative to ER+ and luminal A subtypes, respectively (41). While our sample with data on adipokine receptor gene expression was small and limited our statistical power, larger studies in the future will help clarify these findings. Nonetheless, our findings suggest that distribution of adiposity and adiposity-related expression profiles of the adipokines and adipokine receptors, especially LEP and LEPR, in

TABLE 2 | Multivariable-adjusted associations of body fatness measures with LEP protein and *LEP* gene expression in breast tumor tissues.

	LEP protein expression				*LEP* gene expression			
	n	β (95% CI)	$\beta_{standardized}$	*P*	n	β (95% CI)	$\beta_{standardized}$	*P*
Body mass index (kg/m^2)	635	0.0013 (-0.0007, 0.0033)	0.0650	0.189	144	0.0529 (0.0151, 0.0906)	0.2962	0.007**
Waist circumference (cm)	624	0.0003 (-0.0006, 0.0013)	0.0338	0.507	143	0.0247 (0.0065, 0.0429)	0.2880	0.009*
Hip circumference (cm)	624	0.0006 (-0.0004, 0.0016)	0.0570	0.235	143	0.0259 (0.0066, 0.0453)	0.2684	0.01*
Waist-to-hip ratio	624	-0.0606 (-0.2503, 0.1291)	-0.0328	0.532	143	1.5986 (-2.5554, 5.7526)	0.0880	0.452
Fat mass index (kg/m^2)	594	0.0012 (-0.0019, 0.0044)	0.0405	0.440	136	0.0832 (0.0268, 0.1397)	0.3245	0.005**
Percent body fat (%)	596	-0.0002 (-0.0021, 0.0018)	-0.0103	0.843	136	0.0396 (0.0025, 0.0767)	0.2359	0.038*

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEP as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of LEP as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.
**Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P <0.0083).*

TABLE 3 | Multivariable-adjusted associations of body fatness measures with LEPR protein and LEPR gene expression in breast tumor tissues.

	LEPR protein expression				*LEPR* gene expression			
	n	β (95% CI)	$\beta_{standardized}$	*P*	n	β (95% CI)	$\beta_{standardized}$	*P*
Body mass index (kg/m^2)	571	0.0028 (0.0011, 0.0045)	0.155	0.002**	144	0.0062 (-0.0187, 0.0312)	0.053	0.625
Waist circumference (cm)	561	0.0013 (0.0005, 0.0022)	0.162	0.001**	143	-0.0003 (-0.0122, 0.0116)	-0.005	0.962
Hip circumference (cm)	561	0.0015 (0.0007, 0.0024)	0.163	0.001**	143	-0.0006 (-0.0134, 0.0122)	-0.009	0.927
Waist-to-hip ratio	561	0.0537 (-0.1131, 0.2206)	0.033	0.528	143	-0.1725 (-2.8365, 2.4914)	-0.014	0.899
Fat mass index (kg/m^2)	534	0.0041 (0.0015, 0.0067)	0.159	0.002**	136	0.0091 (-0.0275, 0.0458)	0.055	0.626
Percent body fat (%)	536	0.0020 (0.0004, 0.0036)	0.124	0.016*	136	-0.0012 (-0.0246, 0.0228)	-0.008	0.941

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of LEPR as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.
**Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P <0.0083).*

TABLE 4 | Multivariable-adjusted associations of body fatness measures with ADIPOQ protein and *ADIPOQ* gene expression in breast tumor tissues.

	ADIPOQ protein expression				*ADIPOQ* gene expression			
	n	β (95% CI)	$\beta_{standardized}$	*P*	n	β (95% CI)	$\beta_{standardized}$	*P*
Body mass index (kg/m^2)	618	0.0009 (-0.0013, 0.0032)	0.0405	0.418	144	0.0036 (-0.0515, 0.0587)	0.0140	0.897
Waist circumference (cm)	608	0.0008 (-0.0003, 0.0019)	0.0765	0.137	143	-0.0065 (-0.0328, 0.0198)	-0.0518	0.629
Hip circumference (cm)	608	0.0008 (-0.0004, 0.0019)	0.0654	0.176	143	0.0041 (-0.0241, 0.0323)	0.0291	0.775
Waist-to-hip ratio	608	0.0840 (-0.1239, 0.2919)	0.0414	0.429	143	-5.1582 (-10.9547, 0.6382)	-0.1943	0.083
Fat mass index (kg/m^2)	583	0.0019 (-0.0016, 0.0053)	0.0565	0.285	136	-0.0060 (-0.0890, 0.0770)	-0.0160	0.888
Percent body fat (%)	584	0.0006 (-0.0015, 0.0027)	0.0281	0.589	136	-0.0280 (-0.813, 0.0254)	-0.1144	0.306

*Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of ADIPOQ as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of ADIPOQ as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status. *Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P <0.0083).*

the local organ might have differential impacts on breast cancer based on tumor subtype, and the crosstalk between ER and adipokine biomarkers and other inflammatory biomarkers might play a role (59).

Prior analysis from WCHS reported a lack of association between BMI and breast cancer risk, but higher hip circumference and waist circumference were associated with more than 2-fold increased risk of pre-menopausal breast cancer among women in the fourth quartiles for each measure compared to the first quartile.[42] Further, findings from WCHS also showed that compared to BMI, WHR was more strongly associated with overall and breast cancer-specific mortality among Black women. Specifically, compared to the first quartile, women in the fourth quartile of WHR had 61% and 68% increased risk of overall and breast cancer specific death, respectively, while women with class I and class II obesity (compared to normal weight) had statistically non-significant increased risk of death ranging from 17—33% (44). From the combination of these findings, investigations of the associations between more accurate measures of adiposity and adipose tissue distribution (including overall adiposity, visceral adiposity, and subcutaneous adiposity assessed through computed tomography [CT]), in association with adipokine receptor protein

TABLE 5 | Multivariable-adjusted associations of body fatness measures with ADIPOR1 protein and ADIPOR1 gene expression in breast tumor tissues.

	ADIPOR1 protein expression				*ADIPOR1* gene expression			
	n	β (95% CI)	$\beta_{standardized}$	*P*	n	β (95% CI)	$\beta_{standardized}$	*P*
Body mass index (kg/m^2)	665	-0.0008 (-0.0028, 0.0013)	-0.035	0.473	144	0.0070 (-0.0045, 0.0184)	0.128	0.234
Waist circumference (cm)	654	-0.0002 (-0.0011, 0.0008)	-0.015	0.761	143	0.0048 (-0.0007, 0.0102)	0.181	0.090
Hip circumference (cm)	654	-0.0004 (-0.0014, 0.0007)	-0.032	0.497	143	0.0032 (-0.0032, 0.0086)	0.091	0.367
Waist-to-hip ratio	654	0.0474 (-0.1447, 0.2394)	0.024	0.629	143	1.1785 (-0.0507, 2.4078)	0.212	0.062
Fat mass index (kg/m^2)	624	-0.0019 (-0.0050, 0.0013)	-0.059	0.249	136	0.0081 (-0.0091, 0.0252)	0.104	0.357
Percent body fat (%)	626	-0.0016 (-0.0036, 0.0003)	-0.083	0.099	136	0.0045 (-0.0066, 0.0156)	0.090	0.424

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of ADIPOR1 as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of ADIPOR1 as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

TABLE 6 | Multivariable-adjusted associations of body fatness measures with ADIPOR2 protein and ADIPOR2 gene expression in breast tumor tissues.

	ADIPOR2 protein expression				*ADIPOR2* gene expression			
	n	β (95% CI)	$\beta_{standardized}$	*P*	n	β (95% CI)	$\beta_{standardized}$	*P*
Body mass index (kg/m^2)	584	-0.0003 (-0.0028, 0.0023)	-0.010	0.844	144	0.0098 (-0.0103, 0.0300)	0.100	0.346
Waist circumference (cm)	575	-0.0003 (-0.0015, 0.0009)	-0.029	0.585	143	0.0085 (-0.0012, 0.0182)	0.182	0.088
Hip circumference (cm)	575	-0.0001 (-0.0014, 0.0012)	-0.008	0.873	143	0.0046 (-0.0058, 0.0150)	0.087	0.387
Waist-to-hip ratio	575	-0.0389 (-0.2773, 0.1995)	-0.017	0.749	143	2.1901 (0.0181, 4.3621)	0.221	0.050
Fat mass index (kg/m^2)	552	0.0002 (-0.0037, 0.0040)	0.004	0.939	136	0.0155 (-0.0146, 0.0456)	0.113	0.313
Percent body fat (%)	554	0.0007 (-0.0017, 0.0031)	0.031	0.557	136	0.0136 (-0.0058, 0.0330)	0.151	0.171

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of ADIPOR2 as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Gene expression scores reflect normalized, log2-transformed gene expression of ADIPOR2 as analyzed through the Nanostring nCounter Analysis System. Each model was generated using multiple linear regression adjusting for age, race, menopausal status, and estrogen receptor status.

TABLE 7 | Multivariable-adjusted associations of body fatness measures with LEPR protein expression in breast tumor tissues, stratified by race.

Black women	LEPR protein expression			
	n	β (95% CI)	$\beta_{standardized}$	*P*
Body mass index (kg/m^2)	455	0.0016 (0.0000, 0.0031)	0.0906	0.049*
Waist circumference (cm)	445	0.0006 (-0.0001, 0.0014)	0.0782	0.101
Hip circumference (cm)	445	0.0007 (-0.0001, 0.0015)	0.0791	0.089
Waist-to-hip ratio	445	0.0267 (-0.1276, 0.1810)	0.0169	0.735
Fat mass index (kg/m^2)	428	0.0019 (-0.0042, 0.0042)	0.0791	0.100
Percent body fat (%)	428	0.0008 (-0.0007, 0.0023)	0.0501	0.302
White women	LEPR protein expression			
	n	β (95% CI)	$\beta_{standardized}$	*P*
Body mass index (kg/m^2)	116	0.0050 (0.0011, 0.0089)	0.2303	0.014*
Waist circumference (cm)	116	0.0020 (0.0003, 0.0037)	0.2315	0.022*
Hip circumference (cm)	116	0.0021 (0.0003, 0.0040)	0.2055	0.027*
Waist-to-hip ratio	116	0.1634 (-0.2107, 0.5376)	0.0812	0.394
Fat mass index (kg/m^2)	106	0.0078 (0.0016, 0.0139)	0.2371	0.015*
Percent body fat (%)	108	0.0043 (0.0008, 0.0078)	0.2292	0.018*

*Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Each model was generated using multiple linear regression adjusting for age, menopausal status, and ER status. *Statistically significant at P<0.05.*

and gene expression are critical to elucidating the impact of adiposity on breast carcinogenesis and progression. Such data are critical to determining the clinical utility of adipokine receptor expression profiles as biomarkers of breast cancer risk and prognosis and might contribute to the development of novel interventions and therapeutics targeting high breast tumor expression of these markers, particularly of LEPR, as a means of improving outcomes.

An important strength of this study is that it adds new knowledge regarding the potential impact of overall and central body fatness on adiposity-related biomarkers in breast tumor tissues. Our findings suggest that measures of body fatness are associated with the expression of adipokine receptors – primarily LEP and LEPR – in breast tumors. From this, we generated new hypotheses about the mechanisms linking central adiposity with breast cancer outcomes,

TABLE 8 | Multivariable-adjusted associations of body fatness measures with LEPR protein expression in breast tumor tissues, stratified by menopausal status.

Premenopausal women	**LEPR protein expression**			
	n	**β (95% CI)**	**$\beta_{standardized}$**	***P***
Body mass index (kg/m^2)	264	0.0019 (-0.0002, 0.0040)	0.1090	0.078
Waist circumference (cm)	261	0.0009 (-0.0001, 0.0019)	0.1033	0.096
Hip circumference (cm)	261	0.0007 (-0.0004, 0.0018)	0.0728	0.231
Waist-to-hip ratio	261	0.1446 (-0.0755, 0.3647)	0.0818	0.199
Fat mass index (kg/m^2)	247	0.0026 (-0.0006, 0.0058)	0.1023	0.111
Percent body fat (%)	247	0.0014 (-0.0007, 0.0034)	0.0854	0.183
Postmenopausal women	**LEPR protein expression**			
	n	**β (95% CI)**	**$\beta_{standardized}$**	***P***
Body mass index (kg/m^2)	307	0.0027 (0.0007, 0.0048)	0.1483	0.010*
Waist circumference (cm)	300	0.0010 (0.0001, 0.0020)	0.1244	0.033*
Hip circumference (cm)	300	0.0015 (0.0004, 0.0025)	0.1575	0.005**
Waist-to-hip ratio	300	-0.0420 (-0.2321, 0.1480)	-0.0259	0.665
Fat mass index (kg/m^2)	287	0.0039 (0.0008, 0.0069)	0.1489	0.013*
Percent body fat (%)	289	0.0021 (0.0001, 0.0040)	0.1262	0.036*

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Each model was generated using multiple linear regression adjusting for age, race, and ER status.
**Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P <0.0083).*

TABLE 9 | Multivariable-adjusted associations of body fatness measures with LEPR protein expression in breast tumor tissues, stratified by ER status.

ER+ cases	**LEPR protein expression**			
	n	**β (95% CI)**	**$\beta_{standardized}$**	***P***
Body mass index (kg/m^2)	405	0.0028 (0.0011, 0.0045)	0.1620	0.001**
Waist circumference (cm)	399	0.0014 (0.0006, 0.0022)	0.1712	0.001**
Hip circumference (cm)	399	0.0015 (0.0007, 0.0024)	0.1717	<0.0001**
Waist-to-hip ratio	399	0.0540 (-0.1133, 0.2213)	0.0342	0.527
Fat mass index (kg/m^2)	382	0.0041 (0.0015, 0.0067)	0.1610	0.002**
Percent body fat (%)	385	0.0020 (0.0004, 0.0037)	0.1267	0.017*
ER- cases	**LEPR protein expression**			
	n	**β (95% CI)**	**$\beta_{standardized}$**	***P***
Body mass index (kg/m^2)	166	0.0011 (-0.0018, 0.0040)	0.0613	0.440
Waist circumference (cm)	162	0.0001 (-0.0013, 0.0014)	0.0047	0.954
Hip circumference (cm)	162	-0.0001 (-0.0017, 0.0015)	-0.0105	0.894
Waist-to-hip ratio	162	0.0221 (-0.2635, 0.3076)	0.0132	0.880
Fat mass index (kg/m^2)	152	0.0013 (-0.0026, 0.0053)	0.0546	0.510
Percent body fat (%)	151	0.0011 (-0.0016, 0.0037)	0.0654	0.432

Protein expression scores reflect quantitative protein expression (using immunohistochemistry) of LEPR as analyzed through an automated/unsupervised scoring (quantitative) methodology. The scores estimate the effective staining intensity (ESI) within the effective staining area (ESA) of the biomarker in question (mean±SD of log-transformed values are shown). Each model was generated using multiple linear regression adjusting for age, race, and menopausal status.
**Statistically significant at P<0.05; **Statistically significant with correction for multiple comparisons (P <0.0083).*

which will be pursued. Another strength was the opportunity to perform stratified analysis of the associations of interest by race, menopausal status, and ER status, yielding novel findings. Lastly, our population-based sample that included a large proportion of Black women with breast cancer was also a strength. This study also has some limitations worth noting, including a relatively small sample size (particularly in the gene expression analysis [n = 148]), which may have reduced the power to detect meaningful associations and limit our ability to fully evaluate the complex associations of body fatness with breast cancer. Relatedly, our analysis included multiple comparisons which may have increased the likelihood of observing statistically significant associations. However, we addressed this concern using Bonferroni correction.

Despite these limitations, the findings substantiated our hypothesis that measures of body fatness are associated with expression of adipokine biomarkers in breast tumors. These data are an important step towards understanding the biologic effects of and potential mechanisms linking adiposity with breast cancer risk and prognosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Rutgers University Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AL: Grant funding, study conception and design, data collection, data analysis, data interpretation, and writing. JA: Literature search and data interpretation. T-YC: Data interpretation and manuscript editing. WC: Data collection and manuscript editing. MC: Pathology review, data collection, and manuscript editing. EC: Data interpretation and manuscript editing. BQ: Data collection, data interpretation, and manuscript editing. YL: Data analysis, data interpretation, and manuscript editing. CO: Data interpretation and manuscript editing. TK: Pathology review, data collection, and manuscript editing. C-CH: Grant funding, data collection, and manuscript editing. SY: Data collection, data interpretation, and manuscript editing. CA: Grant funding, data collection, data interpretation, and manuscript editing. EB: Grant funding, data collection, data interpretation, and manuscript editing. KD: Grant funding, data collection, data interpretation, and manuscript editing. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Insulin resistance and inflammation mediate the association of abdominal obesity with colorectal cancer risk

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Background: The close association of abdominal obesity rather than general obesity with colorectal cancer (CRC) risk might be mediated by IR and inflammation, which has never been systematically explored in large-scale prospective studies.

Methods: We prospectively examined the mediation effects of the fasting triglyceride-glucose (TyG) index and C-reactive protein (CRP) on the associations of obesity (general and abdominal) with CRC risk among 93,659 participants. We used the Cox proportional hazards regression models and subgroup analyses to evaluate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) of CRC. The CAUSALMED procedure was used to perform the mediation analyses.

Results: During 13.02 years of follow-up, a total of 586 CRC cases were verified. Male participants with general obesity and abdominal obesity had a 1.29-fold and a 1.28-fold increased risk of CRC. However, a significant association was only observed among female individuals with abdominal obesity. Both TyG index and CRP were associated with an elevated risk of CRC, and A significant interaction between the TyG index and CRP was found for the risk of CRC (P for interaction<0.05). CRP and the TyG index significantly mediated the positive association between abdominal obesity and CRC risk.

Conclusion: CRP and TyG index increased the risk of CRC independently and synergistically. Mediation effects of CRP and the TyG index were found for the association between abdominal obesity and CRC risk.

KEYWORDS

insulin resistance, inflammation, colorectal cancer, obesity, mediation

Introduction

Colorectal cancer (CRC) is deadly and expensive to treat (1). Previous epidemiologic studies have reported a possible association between body size and the risk of CRC (2–6). Body mass index (BMI, in kg/m^2) is positively correlated with CRC risk in men, while women have weaker correlations. In addition, abdominal obesity [as assessed by waist circumference (WC, in cm)] is closely associated with CRC risk in both sexes. One possible explanation for the disparity is that men and women have distinct body compositions, with fat constituting a more significant percentage of body mass in women (30%) than in men (20%) (7). Another explanation might be that abdominal obesity plays a crucial role in metabolic abnormalities, leading to chronic diseases, including cancer (8, 9).

The insulin resistance (IR) and inflammation hypotheses postulate that there is a relationship between abdominal obesity and CRC risk since the buildup of visceral fat is a significant predictor of IR and inflammation (10, 11). IR and inflammation have increased the risk of CRC incidence in experimental and epidemiologic investigations (12, 13). The fasting triglyceride-glucose (TyG) index is a simple and cost-effective way to detect IR (14) compared to the gold standard hyperinsulinemic-euglycemic glucose clamp approach (15). High-sensitivity C-reactive protein (hs-CRP), also known as CRP assessed by high-sensitivity assays, is a typical protein produced in response to inflammation, infection, and tissue damage that has been linked to chronic disorders such as cardiovascular disease (CVD) and cancer (16, 17). Based on the evidence above, we assume that the close association of abdominal obesity rather than general obesity with CRC risk might be mediated by IR and inflammation, which, to our knowledge, has never been systematically explored in large-scale prospective studies.

The Kailuan study is an ongoing, prospective cohort study that includes biennial follow-ups for each participant. The anthropometric and laboratory parameter measurements offer us a valuable opportunity to investigate 1) the association of the TyG index and CRP with the risk of CRC incidence and 2) the mediation effects of the TyG index and CRP on the associations of obesity (general and abdominal) with CRC risk.

Abbreviations: BMI, body mass index; CIs, confidence intervals; CRC, colorectal cancer; CRP, C-reactive protein; CVD, cardiovascular disease; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, hyperinsulinemic-euglycemic glucose clamp; HRs: hazard ratios; TC, total cholesterol; TG, triglyceride; TyG, fasting triglyceride-glucose; WC, waist circumference.

Methods

Study population

As previously stated (17), this study was based on the Kailuan Study, a community-based ongoing cohort study performed in Tangshan city. The current study investigated the risk factors for chronic diseases such as cancer. In short, a sum of 101,510 individuals including 81,110 males and 20,400 females underwent a standardized questionnaire, physical examination, and laboratory testing from June 2006 to October 2007 (baseline). Follow-up examinations were carried out biennially to keep participants up to update participant status on the parameters above.

In this study, individuals were excluded if they 1) were diagnosed with cancer previously(n=377); 2) had missing data on BMI, WC, CRP and the components of the TyG index, including fasting blood glucose (FBG, in mmol/L) and triglycerides (TG, in mmol/L) (n=1,342); and 3) lacked information about any potential confounders, including age, sex, social economic factors, laboratory tests and lifestyle behaviors (n=6,132). After factoring for the exclusion criteria, 93,659 individuals were enrolled, including 18,988 women and 74,671 men (Figure 1).

Laboratory assessments

Patient's venous blood was drawn into EDTA tubes after an overnight fast (8–12 h). All blood samples were analyzed in the Central Laboratory of Kailuan General Hospital using an autoanalyzer (Hitachi 747; Hitachi). The details regarding the assessment of plasma CRP, FBG, HDL-C, TG, and TC can be found elsewhere (18). The TyG index was estimated using the following calculations: ln [TG (mg/dL) × FBG (mg/dL)/2]. According to the Centers for Disease Control and Prevention and the American Heart Association guidelines, low-grade inflammation was defined as CRP ≥ 3 mg/L (19). The median of the TyG index (8.59) was used as the cutoff for the definition of IR.

Ascertaining the outcome

For the duration of the follow-up, incident CRC cases were identified via: 1) tracking participants' biennial health check-ups; 2) examining medical records linked with the Tangshan medical insurance system and the Kailuan Social Security Information System once a year, and 3) checking death certificates from the Provincial Vital Statistics Offices (PVSO) to further confirmed the outcome yearly. Clinical professionals assessed medical records and pathology reports to reconfirm the diagnosis of incident CRC, and CRC patients were categorized as C18-21

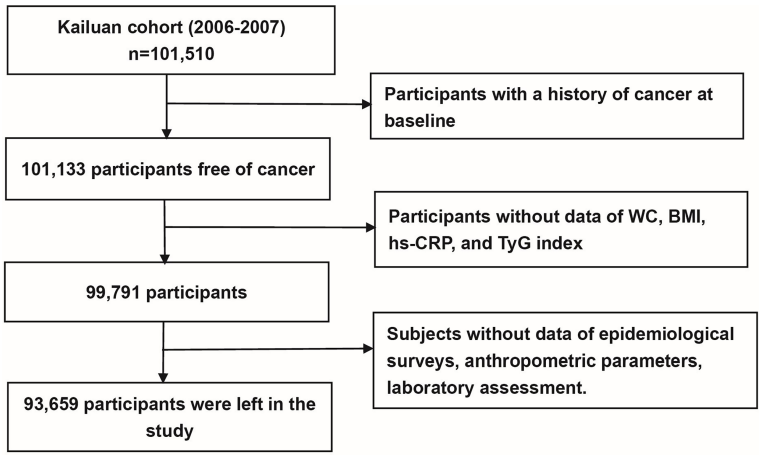

FIGURE 1
Flow chart of study participants.

using the International Classification of Diseases, Tenth Revision (ICD-10).

Potential confounders

Information on age, sex, socioeconomic factors, living habits, and personal and family members' medical histories was collected *via* a standard questionnaire. BMI was calculated as body weight divided by the square of the body height and was grouped into the following three categories: normal (< 24.0 kg/m^2), overweight (24.0-27.9 kg/m^2), and general obesity (≥ 28.0 kg/m^2). Abdominal obesity was defined as WC >90 cm in men and >85 cm in women. Drinking was defined as consuming ≥ 100 mL/day of alcoholic beverages for more than 6 months. Smoking status was defined as consuming ≥ 1 cigarette/day for more than 6 months. Physical activity was defined as having ≥ 3 times weekly with each time lasting ≥ 30 minutes based on the response to the question of frequency of Physical activity. Tea consumption was defined as ≥ 5 times weekly regardless of the tea types. High-fat diets were evaluated in the questionnaire as "Regularly" consumed.

Statistical analysis

The mean ± SD and T-test were used to describe and compare continuous variables in the normal distribution. The median (IQR) and nonparametric tests (Kruskal-Wallis test) were used to describe and compare the skewed distributed variables (e.g., CRP and TG). Absolute values with percentages and the chi-square test were utilized to represent and compare categorical variables. Person-years were computed from the date of the baseline examination to the date of CRC diagnosis, death, or December 31, 2019, whichever occurred first. The hazard ratios (HRs) and 95% confidence intervals (CIs) for the development of incident CRC were calculated using Cox proportional hazards models. We firstly explored the association of general obesity (assessed by BMI) and central obesity (assessed by WC) with subsequent CRC risk among men and women, due to the distinct body compositions between men and women. Secondly, we investigated the effect of IR (assessed by TyG index) and inflammation (assessed by CRP) on the occurrence of CRC, multiplicative models were used to examine the interactions between CRP, TyG index, and CRC risks. Third, because there was an interaction between TyG index, CRP, and CRC risk, participants were further divided into four groups based on the presence/absence of the elevated TyG index (≥ 8.59) and CRP (>1 mg/L).

The CAUSALMED procedure was used to perform the mediation analyses based on the variance-covariance matrix and the maximum likelihood method. It calculated the total effect (the total of the direct and indirect effects), the direct effect (the effect without the mediator's influence), and the indirect effect (the effect of the independent variable on the mediator multiplied the effect of the mediator on the outcome). All *P* values < 0.05 (two-sided) were judged statistically significant. Statistical analyses were carried out using SAS software (SAS Institute, Cary, NC, USA), version 9.4.

Results

The baseline characteristics of the participants stratified by sex are listed in Table 1. The average age of the study population was 51.48 ± 12.44 years. Significant age differences, and levels of

TABLE 1 Baseline characteristics of the participants stratified by sex.

Variables	Overall	Women	Men	*P*-value
n	93,659	18,988	74,671	
Age (year)	51.44 ± 12.45	48.67 ± 11.46	52.15 ± 12.60	<0.001
FBG (mmol/L)	5.48 ± 1.68	5.32 ± 1.64	5.52 ± 1.69	<0.001
HDL-C (mmol/L)	1.55 ± 0.40	1.59 ± 0.39	1.54 ± 0.40	<0.001
TG (mmol/L)	1.27 (0.90,1.93)	1.18 (0.82,1.75)	1.30 (0.92,1.98)	<0.001
TC (mmol/L)	4.95 ± 1.15	4.98 ± 1.09	4.94 ± 1.16	<0.001
CRP (mg/L)	0.80 (0.30,2.06)	0.80 (0.30,2.28)	0.80 (0.30,2.00)	0.002
BMI (Kg/m^2)	25.05 ± 3.50	24.66 ± 3.81	25.15 ± 3.41	<0.001
WC (cm)	86.90 ± 9.99	82.89 ± 10.70	87.92 ± 9.53	<0.001
Per capita income (>800 ¥)	13412 (14.32)	2984 (15.72)	10428 (13.97)	<0.001
Educational background (High school or above, %)	18698 (19.96)	5487 (28.90)	13211 (17.69)	<0.001
Physical exercise (yes, %)	14648 (15.64)	2545 (13.40)	12103 (16.21)	<0.001
Current smoker (%)	28948 (30.91)	268 (1.41)	28680 (38.41)	<0.001
Current drinker (%)	16760 (17.89)	94 (0.50)	16666 (22.32)	<0.001
Family history of cancer (yes, %)	3428 (3.66)	867 (4.57)	2561 (3.43)	<0.001
Diabetes mellitus (yes, %)	8501 (9.08)	1480 (7.79)	7021 (9.40)	<0.001
Hypertension (yes, %)	40861 (43.63)	6075 (31.99)	34786 (46.59)	<0.001
Tea consumption (yes, %)	8818 (9.42)	760 (4.00)	8058 (10.79)	<0.001
Sedentary lifestyle (> 8 h/d, %)	3038 (3.24)	710 (3.74)	2328 (3.12)	<0.001
High-fat diets (regularly, %)	8626 (9.21)	938 (4.94)	7688 (10.30)	<0.001

CRP, high-sensitivity C-reactive protein; BMI, body mass index; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; WC, waist circumference; TC, total cholesterol.

FBG, HDL-C, TC, TG, CRP, BMI, and WC, were found across the different sex groups. In addition, the percentages of the male sex, reported income, marital status, educational levels, physical exercise, tobacco, alcohol and tea consumption, sedentary lifestyle, high-fat diets, hypertension, diabetes mellitus, and family history of cancer differed considerably across the two groups.

During 13 years of follow-up, a total of 586 incident CRC was developed. Table 2 shows the association of general obesity or abdominal obesity with CRC risk. Among the male group, participants with general obesity and abdominal obesity had a 1.29-fold (HR [95%] CI: 1.29, 1.01-1.64) and a 1.28-fold (HR [95%] CI: 1.28, 1.07-1.52) increased risk of CRC. However, a significant association was only observed among female individuals with abdominal obesity (WC $>_{85.0\ \text{vs.}\ \leq 85.0}$, HR [95%] CI: 1.22, 1.03-1.50).

The adjusted HRs (95% CI) for the association of the TyG index and CRP with the risk of CRC are shown in Table 3. TyG index (continuous) and elevated TyG index (≥ 8.59 vs. <8.59) were positively related to the risk of CRC incidence, with

TABLE 2 The association of general obesity or central obesity with CRC risk.

	Men			Women		
	Case/person-years	HR (95%CI)	p-value	Case/person-years	HR (95%CI)	p-value
General obesity[a]						
Normal	174/339508	Ref.		32/111878	Ref.	
Overweight	223/396041	1.10 (0.90,1.34)	0.368	34/84586	1.05 (0.65,1.72)	0.835
Obesity	108/172796	1.29 (1.01,1.64)	0.040	15/41100	0.89 (0.48,1.67)	0.725
Central obesity[b]						
No	281/586301	Ref.		41/151814	Ref.	
Yes	224/322045	1.28 (1.07,1.52)	0.008	40/85750	1.22 (1.03,1.50)	0.013

Adjustments were made for age (every 10 years), family income, educational background, marital status, TC, smoking status, drinking status, physical activity, sedentary lifestyle, tea consumption, salt intake, high-fat diet, hypertension, and family history of cancer.
[a]General obesity was defined as BMI≥28.0 Kg/m^2, and overweight was defined as BMI with a range of 24.0-27.9 Kg/m^2.
[b]Central obesity was defined as WC> 90.0 cm for men, and WC> 85.0 cm for women.

TABLE 3 Hazard ratios (HRs) for the association of TyG index or CRP levels with CRC risk.

Group	Cases/person-years	Crude models		Adjusted models	
		HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
TyG index (continuous)	586/1145910	1.32 (1.17,1.47)	<0.001	1.21 (1.06,1.37)	0.006
TyG index (median)					
< 8.59	228/572835	Ref.		Ref.	
≥ 8.59	358/573075	1.57 (1.33,1.84)	<0.001	1.41 (1.17,1.67)	<0.001
***P* for interaction**[a]					0.048
CRP (continuous)	586/1145910	1.01 (1.00,1.02)	0.128	1.00 (0.99,1.01)	0.758
CRP					
< 1 mg/L	291/672247	Ref.		Ref.	
1-3 mg/L	162/277672	1.36 (1.12,1.65)	0.002	1.17 (0.97,1.43)	0.109
>3 mg/L	133/195992	1.59 (1.29,1.95)	<0.001	1.29 (1.05,1.60)	0.017
***P* for trend**			<0.001		0.042

Adjusted models were adjusted for age (every 10 years), sex, family income, educational background, WC, TC, smoking status, drinking status, physical activity, sedentary lifestyle, tea consumption, high-fat diet, hypertension, diabetes, and family history of cancer.
[a]Interaction between TyG index and CRP for the risk of CRC.

corresponding HRs (95% CI) of 1.21 (1.06-1.37) and 1.41 (1.17, 1.67), respectively. A significant interaction between the TyG index and inflammation (CRP> 3 mg/L) was found for the risk of CRC (*P* for interaction<0.05). There was a statistically significant trend of increasing relative risks of CRC across different CRP groups (CRP $>_{3}$ vs. $_{<1}$, HR [95%] CI: 1.29, 1.05-1.60; p for trend=0.042). Figure 2 illustrates the subgroup analyses stratified by sex, age, abdominal obesity, and diabetes. Significant associations of an elevated TyG index with CRC risk were found among participants who were male, young, middle-aged, elderly, and without abdominal obesity or diabetes. Age significantly modified the associations between CRP and CRC risk (P for interaction < 0.05). The associations were more pronounced among young participants than middle-aged and elderly adults. The positive results were also observed when participants were stratified by sex, abdominal obesity and diabetes.

The significant interaction between the TyG index and inflammation affects CRC development. We further divided participants into four groups based on the absence/presence of

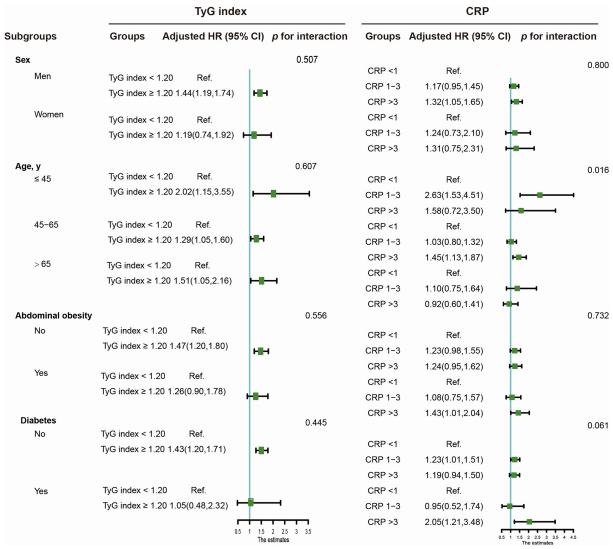

FIGURE 2
Subgroup analysis of the TyG index or CRP level association with CRC risk. Adjusted models were adjusted for age (every 10 years), sex, family income, educational background, marital status, WC, TC, smoking status, drinking status, physical activity, sedentary lifestyle, tea consumption, salt intake, high fat diet, hypertension, and family history of cancer.

TABLE 4 Hazard ratios (HRs) for the association of TyG index and CRP levels with CRC risk.

Group	Cases/person-years	Crude		Adjusted	
		HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
TyG(-) CRP(-) group	179/482505	Ref.		Ref.	
TyG(-) CRP(+) group	49/90337	1.47(1.07,2.02)	0.017	1.25(0.91,1.72)	0.165
TyG(+) CRP(-) group	270/464199	1.57(1.29,1.90)	<0.001	1.42(1.17,1.73)	<0.001
TyG(+) CRP(+) group	88/108870	2.21(1.72,2.86)	<0.001	1.74(1.31,2.28)	<0.001

Adjusted models were adjusted for age (every 10 years), sex, family income, educational background, marital status, WC, TC, smoking status, drinking status, physical activity, sedentary lifestyle, tea consumption, high-fat diet, hypertension, and family history of cancer.
TyG (+): TyG index ≥ 8.59.
CRP (+): CRP ≥ 1 mg/L.

an elevated TyG index and CRP (Table 4). After adjustments were made for the potential confounders, compared with the low TyG index and CRP group, participants with only an elevated TyG index or with an elevated TyG index and CRP had a 1.41-fold (HR [95%] CI: 1.41, 1.16-1.72) and 1.74-fold (HR [95%] CI: 1.74, 1.31-2.28) elevated risk of CRC.

In the mediation effect analysis, both CRP and the TyG index significantly mediated the positive association between abdominal obesity (elevated WC) and CRC risk after adjustments were made for the confounding factors. However, null or weaker mediation effects of the TyG index and CRP were found to associate general obesity with the risk of CRC incidence (Figure 3).

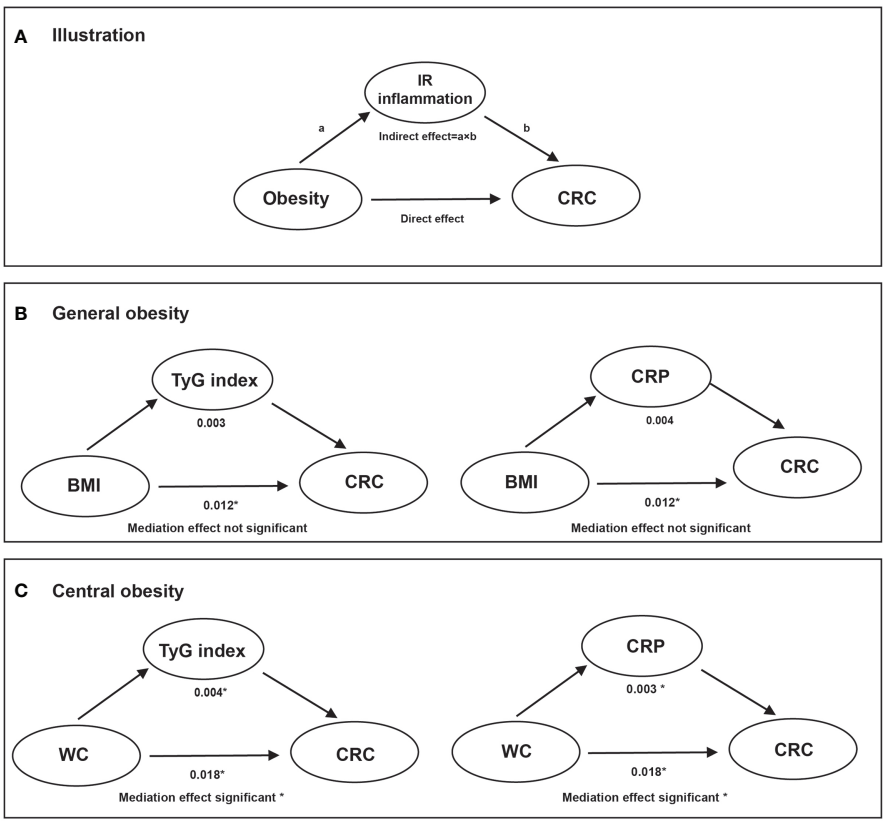

FIGURE 3
The mediation effect of TyG index and CRP on the association of obesity with CRC risk. Note: Adjusted models were adjusted for age (every 10 years), sex, family income, educational background, marital status, WC, TC, smoking status, drinking status, physical activity, sedentary lifestyle, tea consumption, salt intake, high fat diet, hypertension, and family history of cancer. **(A)**: illustration; **(B)**: overall obesity; **(C)**: central obesity.
* Values were statistically significant.

Discussion

In this large-scale community-based cohort study, we found the following: I) abdominal obesity was associated with an elevated risk of CRC in both sexes, while general obesity was found to only increase the risk of CRC in men; II) TyG and CRP could raise the risk of CRC independently. In addition, IR along with inflammation may function synergistically to accelerate the initiation of CRC; III) CRP and TyG index only mediated the association between obesity assessed by WC and CRC risk, indicating the IR and inflammation hypotheses may help to explain the etiological importance of abdominal fat disposition, rather than overall adiposity.

We found that general obesity (BMI> 28 kg/m^2) increased the risk of CRC among male participants, while abdominal obesity (WC>90 cm in men and >85 cm in women) was associated with an elevated risk of CRC incidence in both sexes. This finding is consistent with most previous epidemiological studies (2–6). The close associations between inflammation and CRC incidence have also been demonstrated in previous studies. A report from the general Danish population, which included 10,408 participants, found that elevated levels of CRP in cancer-free individuals were associated with an increased risk of cancer of any type and possibly CRC (20). A case-control study nested within the Japan Public Health Center-based prospective study found a 1.6-fold increased risk of subsequent colon cancer for the highest versus the lowest quartile of CRP (21). A systematic review including 5 nested case-control and 3 cohort studies identified a positive but weak association between pre-diagnostic circulating CRP concentrations and colorectal and colon cancer risk (12).

Little research has been designed to investigate the effect of the TyG index on the occurrence of CRC risk. In a retrospective population-based cohort study of 27,944 individuals, Takuro Okamura et al. found that the TyG index was a useful and accessible tool for predicting incident CRC. Recently, by analyzing 510,471 individuals from six European cohorts, Josef Fritz et al. found that the TyG index was associated with an increased risk of cancers of the digestive system, including colon, rectal, liver, and pancreatic cancer (22). Similarly, several epidemiological studies reported a close association between IR assessed by the homeostasis model assessment method (HOMA-IR) and the risk of colorectal cancer (23, 24), as did experimental studies (25, 26). A study speculated sulphonylureas may play a role in CRC carcinogenesis impairing the physiological insulin secretion among diabetes participants (27).

Epidemiological studies have found that WC is more significantly associated with CRC risk than BMI (28, 29), emphasizing the etiological importance of abdominal fat disposition rather than total adiposity. However, further direct evidence is needed to confirm this association. We found that CRP and the TyG index mediated the association of obesity, assessed by WC rather than BMI, with CRC risk. This finding partly explains the strong association between central obesity and CRC risk, that abdominal obesity-induced carcinogenesis may be through inflammatory and IR pathways. By using UK Biobank data, Dashti SG et al. exanimated the role of obesity-related factors including CRP, hemoglobin-A1c (HbA1c), sex hormone-binding globulin (SHBG), and testosterone in the association of adiposity and CRC risk (30). They found pathways influenced by CRP explained a small proportion of the adiposity-CRC association in both men and women. A prospective cohort study found that substantial proportions of the effect of BMI were mediated by the TyG index for cancers of the pancreas, rectum, colon, kidney, and liver. However, there were two limitations to the previous study. First, it did not explore the significance of those mediation effects. Second, WC was not assessed as an indicator of obesity. Abdominal obesity is a condition marked by low-grade chronic inflammation and IR. Adipose tissue functions as an endocrine organ, secreting a variety of proteins that regulate metabolism, energy intake, and fat storage, including leptin, adiponectin, interleukin-(IL-) 6, and tumor necrosis factor-alpha (TNF-α) (31).

The underlying mechanism by which inflammation and IR increase subsequent CRC risk includes two aspects. First, long-term, low-grade inflammation, which causes protein and DNA damage, can increase tumor growth and progression. Critical pathways that maintain normal cellular homeostasis can be altered by genetic and epigenetic variations in response to inflammatory mediators such as cytokines, free radicals, prostaglandins, and growth factors. Point mutations in tumor suppressor genes, DNA methylation, and posttranslational modifications are examples of these alterations, all of which can contribute to the existence and development of cancer (32). Second, insulin promotes colon cancer progression by increasing the expression of acyl-coenzyme A: cholesterol acyltransferase-1 (33), increasing the expression of vascular cell adhesion molecule-1 in intestinal tumor endothelial cells and causing a proinflammatory state (34), and elevating the levels of IGF-1, which promotes cell proliferation, survival, and angiogenesis by stimulating the synthesis of vascular endothelial growth factor (35).

The main strength of the current study is that it provides a unique perspective on the possible mediation effects of inflammation and IR on the association of obesity with future CRC risk based on a population-based cohort study. Additionally, this study fully considered the influence of potential confounders, such as lifestyle habits and a history of cancer-related illnesses. Additionally, the strengths of this study include the prospective study design, large sample size, and long-term follow-up. Sensitivity analysis and subgroup analyses were conducted to infer the robustness of our conclusion.

There are certain limitations in this study that should be mentioned. First, colon and rectal cancer could not be analyzed separately due to the scarcity of data. Inflammation and IR may have distinct carcinogenic impacts on the development of colon

and rectal cancers. Second, although we controlled for most potential confounders, we could not exclude the possibility of residual cancer-related causal variables, such as the consumption of cereal, vegetable, and high-fiber foods, owing to a lack of information on how these products are consumed. On the other hand, dietary factors are substantially associated with BMI, TC, and TG. Because those factors were adjusted in the multivariate analysis, they may only have had a modest influence on the findings. Third, all participants were from the Kailuan community, with a higher proportion of men than women. As a result, this group could not be considered typical of the Chinese population. The findings could not be immediately extrapolated to other communities with various cultures and socioeconomic backgrounds. Fourth, instead of the gold standard, HOMA-IR, the TyG index was used as an indicator of insulin resistance, which may have resulted in misclassification and underestimation of the potential effect of IR.

Conclusion

The results of this prospective cohort study showed that elevated CRP and TyG index increased the risk of CRC independently and synergistically. Mediation effects of CRP and the TyG index were found for the association between abdominal obesity and CRC risk, which may help to elucidate the etiological importance of abdominal fat disposition rather than overall adiposity.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Aerospace Center Hospital and Kailuan General Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors have read and approved the manuscript. WQL: Methodology, Software, Writing-Original draft preparation. TL: Writing-Reviewing and Editing. LQ: Writing-Reviewing and Editing. YMW: Supervision, Validation. XMM: Software. LYC: Resources. QSZ: Conceptualization, Supervision. JQ: Conceptualization, Supervision, Validation, Resources. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Overweight/obesity-related transcriptomic signature as a correlate of clinical outcome, immune microenvironment, and treatment response in hepatocellular carcinoma

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Backgrounds: The pandemic of overweight and obesity (quantified by body mass index (BMI) ≥ 25) has rapidly raised the patient number of non-alcoholic fatty hepatocellular carcinoma (HCC), and several clinical trials have shown that BMI is associated with the prognosis of HCC. However, whether overweight/obesity is an independent prognostic factor is arguable, and the role of overweight/obesity-related metabolisms in the progression of HCC is scarcely known.

Materials and methods: In the present study, clinical information, mRNA expression profile, and genomic data were downloaded from The Cancer Genome Atlas (TCGA) as a training cohort (TCGA-HCC) for the identification of overweight/obesity-related transcriptome. Machine learning and the Cox regression analysis were conducted for the construction of the overweight/obesity-associated gene (OAG) signature. The Kaplan–Meier curve, receiver operating characteristic (ROC) curve, and the Cox regression analysis were performed to assess the prognostic value of the OAG signature, which was further validated in two independent retrospective cohorts from the International Cancer Genome Consortium (ICGC) and Gene Expression Omnibus (GEO). Subsequently, functional enrichment, genomic profiling, and tumor microenvironment (TME) evaluation were utilized to characterize biological activities associated with the OAG signature. GSE109211 and GSE104580 were retrieved to evaluate the underlying response of sorafenib and transcatheter arterial chemoembolization (TACE) treatment, respectively. The Genomics of Drug Sensitivity in Cancer (GDSC) database was employed for the evaluation of chemotherapeutic response.

Results: Overweight/obesity-associated transcriptome was mainly involved in metabolic processes and noticeably and markedly correlated with prognosis and TME of HCC. Afterward, a novel established OAG signature (including 17

genes, namely, *GAGE2D*, *PDE6A*, *GABRR1*, *DCAF8L1*, *DPYSL4*, *SLC6A3*, *MMP3*, *RIBC2*, *KCNH2*, *HTRA3*, *PDX1*, *ATHL1*, *PRTG*, *SHC4*, *C21orf29*, *SMIM32*, and *C1orf133*) divided patients into high and low OAG score groups with distinct prognosis (median overall survival (OS): 24.87 *vs.* 83.51 months, $p < 0.0001$), and the values of area under ROC curve (AUC) in predicting 1-, 2-, 3-, and 4-year OS were 0.81, 0.80, 0.83, and 0.85, respectively. Moreover, the OAG score was independent of clinical features and also exhibited a good ability for prognosis prediction in the ICGC-LIHC-JP cohort and GSE54236 dataset. Expectedly, the OAG score was also highly correlated with metabolic processes, especially oxidative-related signaling pathways. Furthermore, abundant enrichment of chemokines, receptors, MHC molecules, and other immunomodulators as well as PD-L1/PD-1 expression among patients with high OAG scores indicated that they might have better responses to immunotherapy. However, probably exclusion of T cells from infiltrating tumors resulting in lower infiltration of effective T cells would restrict immunotherapeutic effects. In addition, the OAG score was significantly associated with the response of sorafenib and TACE treatment.

Conclusions: Overall, this study comprehensively disclosed the relationship between BMI-guided transcriptome and HCC. Moreover, the OAG signature had the potential clinical applications in the future to promote clinical management and precision medicine of HCC.

KEYWORDS

hepatocellular carcinoma, overweight, machine learning, signature, genomic alteration, immune microenvironment, sorafenib, TACE

1 Introduction

Hepatocellular carcinoma (HCC), accounting for 75%–80% of primary liver cancer, is the seventh most common cancer and occupies nearly 8.0% of all cancer-related deaths, with more than 0.9 million new cases and 0.8 million deaths worldwide (1). Currently, surgical resection and liver transplantation remain the most effective therapy for HCC patients, but most patients with advanced diseases are not suitable for surgeries (2). Despite receiving surgical treatments, 5-year overall survival (OS) rate of HCC is still poor, and relapse and metastasis rates are quite high (3). With the rapid development of sequencing technologies, comprehensive analysis of molecular characterizations offers novel insights into HCC carcinogenesis and reveals exogenous/endogenous factors potentially influencing HCC progression (4). More importantly, molecular subtyping could divide patients into different HCC subclasses with distinct prognoses, molecular features, and treatment responses altogether, which would help promote the clinical management of HCC patients and select suitable treatment regimens.

So far, HCC has been documented as a cancer type presenting a highly close relationship between tumors and environmental agents. In addition to genetic predisposition, etiological risk factors of chronic hepatitis B/C virus (HBV/HCV) infection, alcohol, tobacco smoking, obesity, contaminants/toxins, and diabetes are frequently reported to induce tumorigenesis of HCC (5). Generally, HBV/HCV-induced HCC originates from chronic liver damage, and HBV/HCV-encoded proteins could alter host transcriptome, progressively stimulating HCC cell proliferation, angiogenesis, invasion, metastasis, and reprogramming cell metabolism (6). Noticeably, alcohol consumption or abuse can greatly increase the risk for HCC, irrespective of whether concomitant HBV/HCV infection or not (7). Moreover, alcohol-related HCC patients have a worse prognosis when compared with those with non-alcoholic HCC (8). Molecular characterizations of alcohol-related HCC subtype have been intensely looked into, and some alcohol-related molecular features may serve as potential diagnostic/prognostic biomarkers or molecular targets, especially alcohol-associated metabolites (9) and alcohol metabolism-associated genes/enzymes (10) highly correlated with HCC morbidity and/or mortality. Undoubtedly, cigarette smoking is associated with a high risk of HCC; as acknowledged, smoke/nicotine exposure can aggravate HCC inflammation,

suppress the anti-tumor effect of T cells, and stimulate cancer stem cell epithelial-to-mesenchymal transition, and smoke and other risk factors positively interact in the development and progression of HCC (11). A population-based study further displays that the OS time of HCC patients varies as a consequence of distinct etiological risk factors because these etiological risk factors could determine a unique molecular profile (12). Due to the epidemic of overweight/obesity over past decades, excess body weight has emerged as a closely relevant risk factor for HCC, and body mass index (BMI) is found to be positively correlated with the mortality rate of liver cancer in both men and women (13). In addition to hyperlipidemia/hypertension, metabolic syndrome, and diabetes, overweight/obesity or higher BMI becomes one of the major risk factors for non-alcoholic fatty liver disease (NAFLD), which is highly correlated with the development of HCC, particularly within those having NAFLD-related cirrhosis and fibrosis (14). Moreover, approximately 20%–30% of NAFLD-related HCC cases develop into HCC in the absence of cirrhosis and fibrosis, and NAFLD is a leading cause of HCC in the absence of cirrhosis and fibrosis (15). Overall, increasing pieces of evidence have disclosed the relationship between overweight/obesity (or high BMI) and tumor progression; however, comprehensive molecular characterizations related to overweight/obesity (or high BMI) in HCC remain to be fully elucidated.

The present study is the first time to reveal that overweight/obesity-related transcriptomic features could distinguish HCC patients with distinct prognoses, biological metabolism, and the immune microenvironment. Based on this overweight/obesity-related transcriptome, a novel overweight/obesity-associated gene (OAG) signature together with a scoring system was subsequently constructed. From a new perspective, the underlying signaling pathways, genomic alterations, and tumor microenvironment were deeply investigated in HCC. Intriguingly, the OAG score was also found to be closely correlated with sorafenib and transcatheter arterial chemoembolization (TACE) treatment responses; furthermore, the OAG score was also of guiding significance to evaluate chemotherapy response.

2 Materials and methods

2.1 Data collection and preprocessing

In the present study, clinical information, mRNA expression data, and genomic data of 360 HCC patient samples (cases without complete information were excluded) were retrieved from The Cancer Genome Atlas (TCGA) *via* the cBioPortal (https://www.cbioportal.org/), regarded as the training cohort. As mentioned earlier, alcohol consumption might aggravate the development and progression of HCC; thus, patients with a risk history of alcohol consumption were excluded. The remaining 199 patient samples were collected to explore the relationship between overweight/obesity and the OS of HCC patients and identify the differentially expressed genes (DEGs) between patients presenting with overweight/obesity or not. In addition, a total of 232 HCC patient samples from the International Cancer Genome Consortium (ICGC; https://dcc.icgc.org/projects/LIRI-JP, namely, ICGC-LIHC-JP) and 72 patient samples selected from the GSE54236 dataset in the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/), respectively, were downloaded as two independent validation cohorts.

2.2 Overweight/obesity-associated transcriptome and unsupervised hierarchical clustering analysis

Of the selected 199 HCC patients in the training cohort, 91 and 108 cases had BMI over 25 and below 25, respectively. The mRNA expression data, with the format of fragments per kilobase million (FPKM), were initially normalized by $\log_2$ (FPKM + 0.001) and then utilized for the DEG analysis ($p < 0.05$, $|\log_{1.5}$ (fold change)$| > 1$) between patient samples with BMI over 25 and below 25, by using the package "DeSeq2". The result of the DEG analysis was exhibited *via* the volcano plot by using the package "ggplot2". Based on the overweight/obesity-derived DEGs, which were also defined as the integrated overweight/obesity-associated transcriptome, unsupervised hierarchical clustering separated this part of HCC patients into different clusters by using the package "Fastcluster". The Kaplan–Meier curve analysis was conducted to compare the OS of different clusters by using the package "survival". Similarly, in the whole TCGA-HCC cohort, unsupervised hierarchical clustering by a foundation of overweight/obesity-associated transcriptome also distinguished two clusters (clusters 1 and 2), and the principal component analysis (PCA) was conducted to display the discrepancy of these two clusters by using the package "ggbiplot".

2.3 Functional enrichment analysis

Based on the DEGs between different clusters, Gene Ontology (GO; http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.kegg.jp/) pathway enrichment analyses (16, 17) were performed by using the package "clusterProfiler" to exhibit the biological activities underlying overweight/obesity-associated transcriptome.

2.4 Tumor microenvironment evaluation

Additionally, tumor purity, ESTIMATE, and TIDE scores were employed to evaluate the tumor microenvironment (TME) of these two clusters (18, 19). Based on the bulk mRNA expression data, a total of 122 immune-related modulators, including chemokines, MHC molecules, receptors, and other immunomodulators, were retrieved to estimate the immunological characteristics (20). The expression of 122 immunomodulators was exhibited by using the package "pheatmap". The cancer immunity cycle, containing seven steps and reflecting the anti-cancer immune response, was used to determine the activities of anti-cancer immunity (21). The single-sample gene set enrichment analysis (ssGSEA) was conducted to characterize the activity of each step (22). Finally, multiple kinds of immune checkpoint gene expression profiles were investigated (23).

2.5 Machine learning for the construction of a novel OAG signature

Initially, mRNA expression data of HCC tumor and normal samples were downloaded from the data portal of UCSC xena (https://xenabrowser.net/datapages/) to identify HCC-associated DEGs. Next, the overlapping gene set between overweight/obesity-associated DEGs and HCC-associated DEGs was collected, which was visualized in a Venn plot by using the package "eulerr". The overlapping genes were then enrolled into the univariate Cox regression analysis by using the package "rms" to screen out the OS-related genes. Subsequently, the random forest (RF) algorithm was used to select the representative genes (normalized variable importance measure index > 0.40) by using the package "randomSurvivalForest". Based on the expression of representative genes, the least absolute shrinkage and selection operator (LASSO) Cox regression analysis was conducted to construct a novel OAG signature by using the package "glmnet"; correspondingly, the OAG score of each sample was calculated by the following formula:

$$OAGs\ score = \sum_{x=1}^{n} OAG_x * Coef_x$$

where n, OAG_x, and $Coef_x$ represent the number of OAGs included in the signature, OAG expression level, and coefficient value, respectively.

In TCGA-HCC cohort, patients were assigned to the high and low OAG score groups according to the median OAG score as the cutoff value. The Kaplan–Meier curve analysis was conducted to compare the OS between these two groups. The receiver operating characteristic (ROC) curve analysis, quantified by the value of area under the ROC curve (AUC), was utilized to evaluate the performance of the OAG score in prognosis prediction by using the package "rms". In addition, the Kaplan–Meier curve and ROC curve analysis were also conducted in the ICGC-LIHC-JP cohort and GSE54236 dataset to validate the robustness of the OAG score in prognosis prediction. Furthermore, we compared the predictive accuracy of the OAG signature with other risk signatures, including immune- (24), mitochondrial- (25), energy metabolism- (26), ferroptosis- (27), cuprotosis- (28), and TGF-β-related (29) signatures. The univariate and multivariate Cox regression analyses were conducted to recognize whether the OAG score was an independent prognostic factor.

2.6 Single OAG analysis and immunohistochemistry staining

Regarding the role of single OAG expression in HCC, the heatmap plot demonstrated the detailed information of each signature-related OAG expression and corresponding clinical features in samples from TCGA-HCC cohort. Moreover, Pearson's correlation analysis was conducted to investigate the correlation of each OAG expression. Underlying a single OAG expression, the Kaplan–Meier curve analysis was conducted to exhibit the prognostic significance of OAGs; meanwhile, the ROC curve analysis was also performed for each OAG. Eventually, OAG protein expression was analyzed by immunohistochemistry (IHC) staining using the available HCC tumor macro-array staining from the Human Protein Atlas (https://www.proteinatlas.org/). Collectively, 10–12 HCC samples were analyzed for the expression of DPYSL4, MMP3, HTRA3, PDX1, C21orf29, ATHL1, PDE6A, DCAF8L1, SLC6A3, and RIBC2 proteins, while there was no information of IHC staining for the expression of GABRR1, GAGE2D, KCNH2, PRTG, SHC4, and SMIM32 proteins. Also, there was no IHC staining information on *C1orf133*, which was a kind of non-coding RNA (ncRNA).

2.7 Molecular characterizations associated with OAG score

Based on the DEGs between the high and low OAG score groups, GO and KEGG pathway enrichment analyses were initially conducted to identify the critical biological activities/pathways associated with the OAG score. First, the gene set enrichment analysis (GSEA) was performed by using the package "GSVA", and Hallmark gene sets were obtained for GSEA (https://www.gsea-msigdb.org/gsea/msigdb/genesets.jsp?collection=H). Second, genomic alteration data in TCGA-HCC cohort was employed to visualize the discrepancy between the

high and low OAG score groups by using the package "maftools"; meanwhile, the CoMEt algorithm was utilized to investigate the co-occurrence and mutually exclusive alterations (30). The specific alteration sites of the prevalent genes were exhibited *via* the lollipop plot. Same as described before, TME characteristics associated with OAGs were lastly investigated by the following indexes: tumor purity, ESTIMATE, TIDE, and the infiltration of 22 immune cells. Immunological characteristics of immunomodulators, cancer immunity cycle, and immune checkpoint gene expression associated with the OAG score were also compared between the high and low OAG score groups.

2.8 Estimate of treatment responses by sorafenib, TACE, and chemical drugs

As known, sorafenib, TACE, and chemotherapeutic treatments are usually selected for HCC patients. GSE109211 dataset (31), composed of 21 responders and 46 non-responders (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE109211) when receiving sorafenib treatment, was downloaded to explore whether the OAG score or OAG expression was correlated with sorafenib treatment response in HCC. Subsequently, the GSE104580 dataset (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE104580) of 147 HCC patients treated with TACE treatment, including 81 responders and 66 non-responders, respectively, was retrieved to investigate the correlation between the OAG score or OAG expression and response to TACE treatment. In addition, the Genomics of Drug Sensitivity in Cancer (GDSC; https://www.cancerrxgene.org/) database of pharmacogenomic data was downloaded to calculate the half-maximal inhibitory concentration (IC50 value), which was used for chemotherapeutic response prediction. In the present study, cisplatin, 5-fluorouracil (5-FU), paclitaxel, vinblastine, and other commonly used chemical drugs were evaluated.

2.9 Statistical analysis

All statistical analyses were conducted in the present study *via* the R software (version 4.1.1). Fisher's exact test and Student's t-test were used for comparisons of categorical variables and continuous variables. Moreover, the Wilcoxon test and Kruskal–Wallis test were applied for comparisons between two and multiple comparisons. The Kaplan–Meier curve analysis was conducted using the log-rank test. The univariate and multivariate Cox regression analyses were used to disclose the factors associated with survival. The correlation between variables was calculated by using Pearson's coefficient. The significant difference was considered with at least $p < 0.05$. The overall study design is shown in Figure 1.

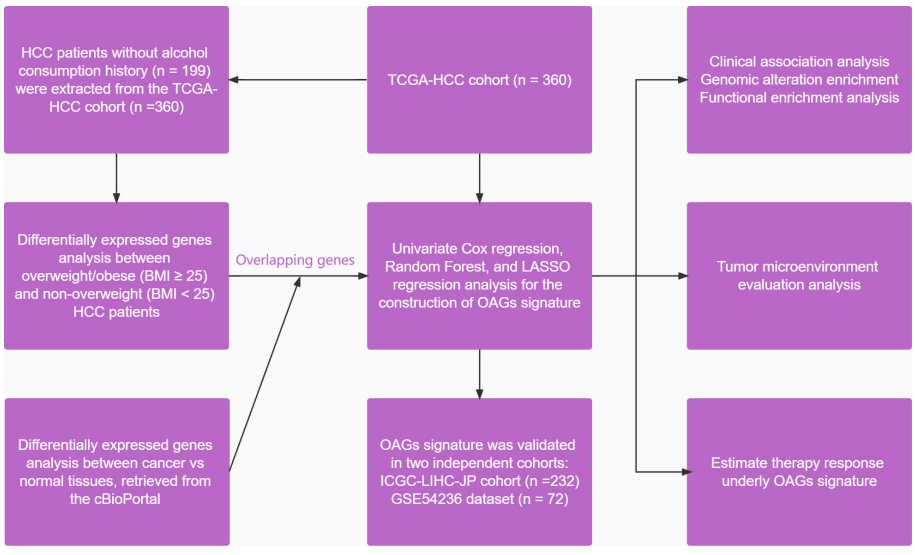

FIGURE 1
A flowchart of study design in the present study.

3 Results

3.1 Identification of overweight/obesity-associated transcriptome among patients not using alcohol

As previously described, alcohol consumption significantly increased the risk of HCC; correspondingly, HCC-related symptoms aggravated gradually. In line with previous findings, we did not observe any significant difference in OS between HCC patients with distinct BMI in the whole TCGA-HCC cohort (Table 1; Figure S1A). As those patients with alcohol consumption were excluded (Table S1), intriguingly, remaining HCC patients with overweight/obesity (BMI ≥ 25) tended to have a worse OS (median OS, 51.25 months *vs.* unreached, p = 0.34, Figure S1B). Between HCC patients with BMI ≥25 and <25, a total of 882 DEGs were identified ($p < 0.05$, $|\log_{1.5}(\text{fold change})| > 1$, Figure S1C), and these DEGs were mainly enriched in the biological activities of metabolic processes, oxygen transport, stem cell proliferation, and WNT protein binding (Figure S1D). Based on the expression of 882 DEGs, unsupervised hierarchical clustering analysis (Figure S1E) identified two subgroups with distinct OS (median OS, 51.25 months *vs.* unreached, p = 0.0019, Figure S1F) among HCC patients without alcohol consumption.

3.2 Overweight/obesity-associated transcriptome and functional annotation

When the whole TCGA-HCC cohort was considered as a training cohort, the overweight/obesity-associated transcriptome also differentiated two clusters (Figure 2A) with significantly different OS (median OS: cluster 1 *vs.* cluster 2, 46.75 months *vs.* 81.67 months, p = 0.032, Figures 2B, C), and there was a higher proportion of patients with overweight/obesity in cluster 1 (p = 0.262, Figure 2D). Functional enrichment revealed that overweight/obesity-associated transcriptome was highly correlated with fatty acid metabolism, cytochrome P450-mediated metabolism, oxidative signaling pathways, and multiple cancer-related metabolisms (Figure S2). Furthermore, no significant difference in tumor mutational burden (TMB) was observed between the two clusters (Figure 2E), but it was noticeable that cluster 1 had higher ESTIMATE and TIDE scores but lower tumor purity (Figures 2F–H). In addition, a large number of chemokines, receptors, MHC molecules, and immunomodulators (Figure 2I) as well as the effector genes of CD8+ T cells, dendritic cells, macrophages, NK cells, and Th1 cells were upregulated in cluster 1 (Figure 2J). Correspondingly, activities of Steps 1 (release of cancer cell antigens) and 4 (trafficking of immune cells tumors) were upregulated in cluster 1; however, activities of Steps 2 (cancer antigen presentation), 6 (recognition of cancer cells by T cells), and 7 (killing of cancer cells) were downregulated (Figure 2K), while the expression of most immune checkpoint genes, including *PD-L1*, *PD-1*, *CTLA-4*, *LAG-3*, *TIGIT*, *TIM-3*, *CD80*, *CD200*, and *CD276*, was markedly upregulated, but only the expression of *PVR* was downregulated in cluster 1 (Figure 2L).

3.3 A novel OAG score as correlate of prognosis of HCC patients

Given overweight/obesity-associated metabolic transcriptome, RF algorithm and LASSO Cox regression analysis were conducted to construct an OAG signature. Initially, it was discovered that 543 of 882 OAGs were differentially expressed between normal and tumor samples (Figure 3A; Table S2), among which the expression of 262 OAGs was significantly correlated with OS of HCC patients in TCGA-HCC cohort (Table S3). Next, the RF algorithm screened out the most representative 26 OAGs (Figures 3B, C). After the over-fitting by the LASSO Cox regression analysis was minimized, a novel signature consisting of 17 OAGs together with an OAG signature scoring system was constructed (Figure 3D; Table 2). According to the median cutoff value, the OAG score separated TCGA-HCC cohort population into two distant groups, termed the high and low OAG score groups. Comparatively, the high OAG score group had quite worse OS (median OS, 24.87 *vs.* 83.51 months, p < 0.0001, Figure 3E). Noticeably, the AUC values of the OAG score in predicting 1-, 2-, 3-, and 4-year OS were 0.81, 0.80, 0.83, and 0.85, respectively (Figure 3F), suggesting that a novel OAG signature performed well in prognosis prediction. Subsequently, the OAG signature was further verified in two independent cohorts, ICGC-LIHC-JP cohort (Table S4) and GSE54236 dataset (Table S5), and indeed, it was observed that the OAG score was negatively correlated with OS (median OS in ICGC-LIHC-JP cohort: unreached *vs.* unreached, p = 0.0004; GSE54236 dataset, 16.98 *vs.* 28.01 months, p < 0.0001, Figures 3G–J). Within these two independent validation cohorts, almost all AUC values of the OAG score in predicting OS were relatively high, confirming that the OAG signature is reliable and robust in prognosis prediction. When being compared with already reported prognostic signatures, such as immune, mitochondria, energy metabolism, ferroptosis, cuprotosis, and TGF-β related signatures, the OAG signature outperformed in prognosis prediction (Figure S3). For HCC patients without alcohol consumption, the OAG signature seemed to perform better in prognosis prediction (Figure S4), and the high OAG score group had a higher proportion of patients with overweight/obesity (51.01% *vs.* 41.10%, p = 0.215) when compared with the low OAG score group.

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TABLE 1 Patient characteristics in TCGA-HCC cohort.

Features		Number
Total		360
Age	Median (range)	61 [16, 90]
Gender	Male	242
	Female	118
Alcohol use	Used	161
	Other	199
Body mass index	<25	173
	≥25	154
Vascular invasion	Macro	16
	Micro	89
	None	202
Histological grading	G1	54
	G2	171
	G3	118
	G4	12
T stage	T1	177
	T2	90
	T3	77
	T4	13
	NA	3
N stage	N0	247
	N1	3
M stage	M0	260
	M1	3
Clinical stage	I	169
	II	84
	III	82
	IV	4
HBV/HCV status	HBV positive	94
	HCV positive	211
	HBV and HCV positive	7
	HBV and HCV negative	48

HBV/HCV, hepatitis B/C virus.

3.4 Prognostic significance and contribution of OAGs

Overall, there were 11 and 6 OAGs serving as OS-related risk factors and protective factors, respectively (Figure 4A). In TCGA-HCC cohort, the expression of *GAGE2D*, *PDE6A*, *GABRR1*, *DCAF8L1*, *DPYSL4*, *SLC6A3*, *MMP3*, *RIBC2*, *KCNH2*, *HTRA3*, and *PDX1* remarkably increased in the high OAG score group, and the expression of each OAG was positively associated with the OAG score, whereas the

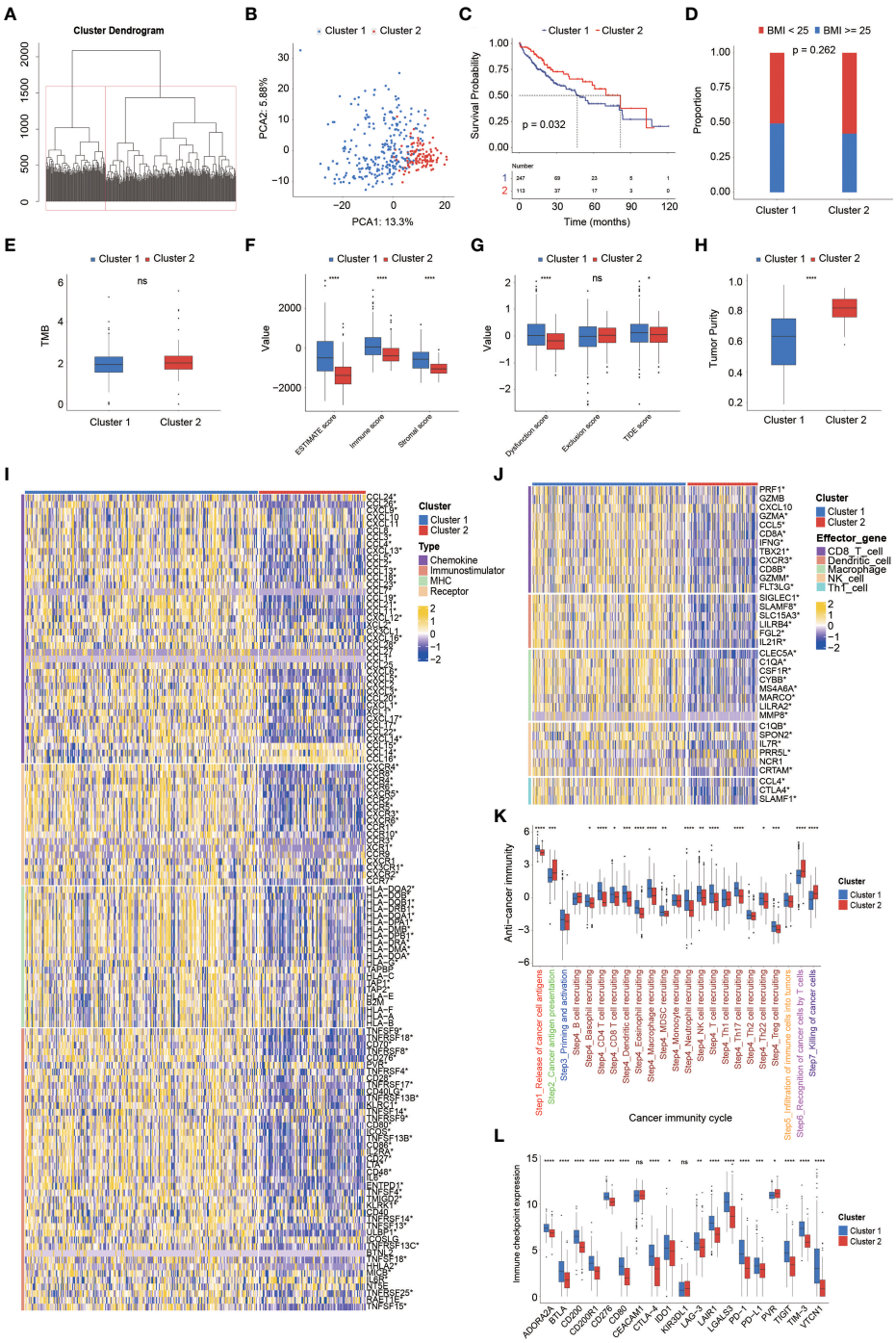

FIGURE 2
The overweight-associated transcriptome highly correlated with prognosis, immune characteristics, and anti-cancer immunity in TCGA-HCC cohort. **(A)** Unsupervised hierarchical clustering by foundation of overweight-associated transcriptome in TCGA-HCC cohort. **(B)** Principal component analysis for two clusters. **(C)** Kaplan–Meier curve analysis for two clusters. **(D)** Proportional analysis of patients with overweight/obesity between these two clusters. **(E–H)** Comparison of TMB level **(E)**, ESTIMATE score **(F)**, TIDE score **(G)**, and tumor purity **(H)** between clusters 1 and 2. **(I)** Differences in the expression of immunomodulators (chemokines, receptors, MHC molecules, and other immunomodulators) between clusters 1 and 2. **(J)** Evaluation of effector gene expression of tumor-infiltrating immune cells. **(K)** Comparison of cancer immunity cycles between clusters 1 and 2. **(L)** Comparison of immune inhibitory checkpoint expression between clusters 1 and 2. TMB, tumor mutational burden.

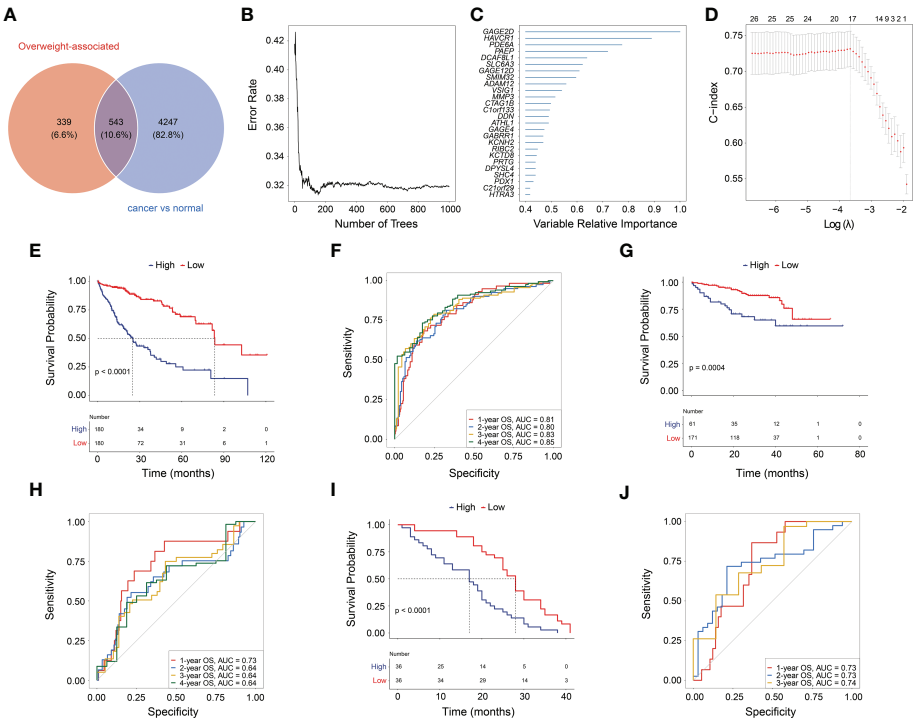

FIGURE 3
Machine learning for construction of overweight-associated gene (OAG) signature. **(A)** Overlapping of overweight-associated genes and differentially expressed genes between cancer and normal samples. **(B)** The random forest algorithm for identification of key genes correlated with overall survival (OS) in HCC. **(C)** The evaluation of the importance of selected OAGs. **(D)** The least absolute shrinkage and selection operator (LASSO) Cox regression analysis for determining an OAG signature and corresponding scoring system. **(E)** Kaplan–Meier curve analysis between high and low OAG score in TCGA-HCC cohort. **(F)** The receiver operating characteristic (ROC) curve analysis of OAG signature in prognosis prediction in TCGA-HCC cohort. **(G)** Kaplan–Meier curve analysis between high and low OAG score in ICGC-LIHC-JP cohort. **(H)** The ROC curve analysis of OAG signature in prognosis prediction in ICGC-LIHC-JP cohort. **(I)** Kaplan–Meier curve analysis between high and low OAG scores in GSE54236 dataset. **(J)** The ROC curve analysis of OAG signature in prognosis prediction in GSE54236 dataset. HCC, hepatocellular carcinoma.

expression of *ATHL1*, *PRTG*, *SHC4*, *C21orf29*, *SMIM32*, and *C1orf133* (ncRNA) elevated in the low OAG score group, and their expression was negatively associated with the OAG score (Figures 4A, B). As expected, the overexpression of 11 risk-related OAGs indicated poorer OS, but the overexpression of six protective-related OAGs was correlated with prolonged OS in HCC ($p < 0.05$, Figure 4C). It was noteworthy that the AUC values of *ATHL1*, *GAGE2D*, and *RIBC* in predicting 1-, 2-, 3-, and 4-year OS were all beyond 0.60, although the predictive ability of single OAG was inferior to that of the OAG signature (Figure S5). In addition, it was observed that 11/11, 11/11, 4/12, 2/12, 1/11, and 12/12 HCC samples expressed DPYSL4, MMP3, HTRA3, PDX1, C21orf29, and ATHL1 proteins in the cytoplasm/membrane, respectively (Figure 4D), but the IHC staining of PDE6A (0/11), DCAF8L1 (0/11), SLC6A3 (0/11), and RIBC2 (0/10) was negative. In contrast, in the Human Protein Atlas (HPA) database, there was no information on the IHC staining of GABRR1, GAGE2D, KCNH2, PRTG, SHC4, and SMIM32. Moreover, *C1orf133* belonged to ncRNA.

3.5 Association between independent OAG score and clinical features

A combination of univariate and multivariate Cox regression analyses revealed that the OAG score was a robust prognostic factor (HR = 5.20, 95% CI, 2.55–10.60, $p < 0.0001$), which was independent of clinical features in TCGA-HCC cohort (Table 3). More importantly, the OAG score was a better predictor of OS for HCC patients than the baseline clinical characteristics (Figure S6). Regarding the relationship between an independent OAG score and clinical features, the high OAG score group had more HCC patients with higher alpha-fetoprotein (AFP) levels ($p < 0.01$), T stages ($p < 0.01$), clinical stages ($p < 0.05$), grades ($p < 0.05$), and macro- or micro-vascular invasions but a lower proportion of HBV-infected HCC patients ($p < 0.001$, Figure S7). Correspondingly, the OAG score was positively correlated with AFP level ($p < 0.001$), T stage ($p < 0.001$), clinical stage ($p < 0.001$), high grade ($p < 0.05$), and vascular invasion, and HBV-infected HCC patients had the

TABLE 2 A total of 17 genes included in overweight-associated genes signature.

Gene name	HR	95% CI	Coefficient
ATHL1	0.5179	0.3643–0.7362	−0.1429
SMIM32	0.4909	0.3438–0.7009	−0.1096
PRTG	0.5421	0.3770–0.7794	−0.0026
SHC4	0.5573	0.3888–0.7988	−0.0411
C21orf29	0.5339	0.3666–0.7775	−0.0306
C1orf133	0.4811	0.3293–0.7026	−0.1294
MMP3	2.3626	1.6101–3.4668	0.0305
GABRR1	2.1684	1.5228–3.0877	0.1039
GAGE2D	2.7373	1.8823–3.9806	0.1021
DPYSL4	2.2922	1.3895–3.7813	0.0307
SLC6A3	1.7895	1.2593–2.5429	0.0086
RIBC2	1.6269	1.1503–2.3011	0.0086
DCAF8L1	1.6812	1.1560–2.4449	0.0157
PDE6A	1.5970	1.0556–2.4161	0.1873
KCNH2	1.5493	1.0335–2.3225	0.0740
HTRA3	1.6280	1.1363–2.3324	0.0641
PDX1	1.4462	1.005–2.0814	0.0439

lowest OAG score ($p < 0.001$, Figure S8). Owing to a limited number of patients with lymph node metastasis or distant metastasis, there was no discrepancy in the OAG score between N0 and N1+ stage groups or M0 and M1 stage groups. As for distinct HCC subtypes that were separated by these baseline clinical features, the OAG score still exhibited excellent performance in prognosis prediction, and the high OAG score group always had an inferior OS ($p < 0.05$, Figure S9). Moreover, stratification analysis demonstrated that the OAG score could potentially predict prognosis for early-stage HCC patients.

3.6 OAG score-associated tumors with different metabolic characteristics

A total of 2,502 DEGs ($p < 0.05$, $|\log_{1.5}(\text{fold change})| > 1$, Table S6) were identified between the high and low OAG score groups. These genes were subsequently enrolled into functional enrichment analysis to evaluate the differential biological activities and signaling pathways between the high and low OAG score groups. GO and KEGG pathway enrichment analyses showed that fatty acid metabolism, cytochrome P450-mediated metabolism, amino acid metabolism, retinol metabolism, and xenobiotic metabolism were majorly involved (Figures 5A, B). Noticeably, GO and KEGG pathway enrichment analyses underlying a single OAG resulted in similar findings (Table S7). The GSEA of Hallmark pathways revealed that oxidative phosphorylation and cell cycle/DNA replication-related signaling pathways, including G2M checkpoint, E2F targets, and mitotic spindle, were significantly enriched in the high OAG score group (Figure 5C), whereas bile acid and xenobiotic metabolism were suppressed in the high OAG score group.

3.7 OAG score associated with distinct somatic genome

Likewise, there was no significant association between the OAG score and TMB level (Figure 6A). Based on the whole-exome sequencing (WES) data from TCGA-HCC cohort, it was identified that 52 genes and 52 genes were altered in more than 5% of patient samples in the high and low OAG score groups, respectively (Table S8). Subsequently, oncoprint plots illustrated the top 20 most prevalently altered genes in the corresponding groups (Figures 6B, C). Collectively, most genomic alterations were missense; meanwhile, *TP53*, *TTN*, and *CTNNB1* occupied the top three positions in both groups. Based on the top 20 most frequently altered genes in the high and low OAG score groups, it was found that co-occurrence landscapes were distinct between the high and low OAG score groups (Figures 6D, E),

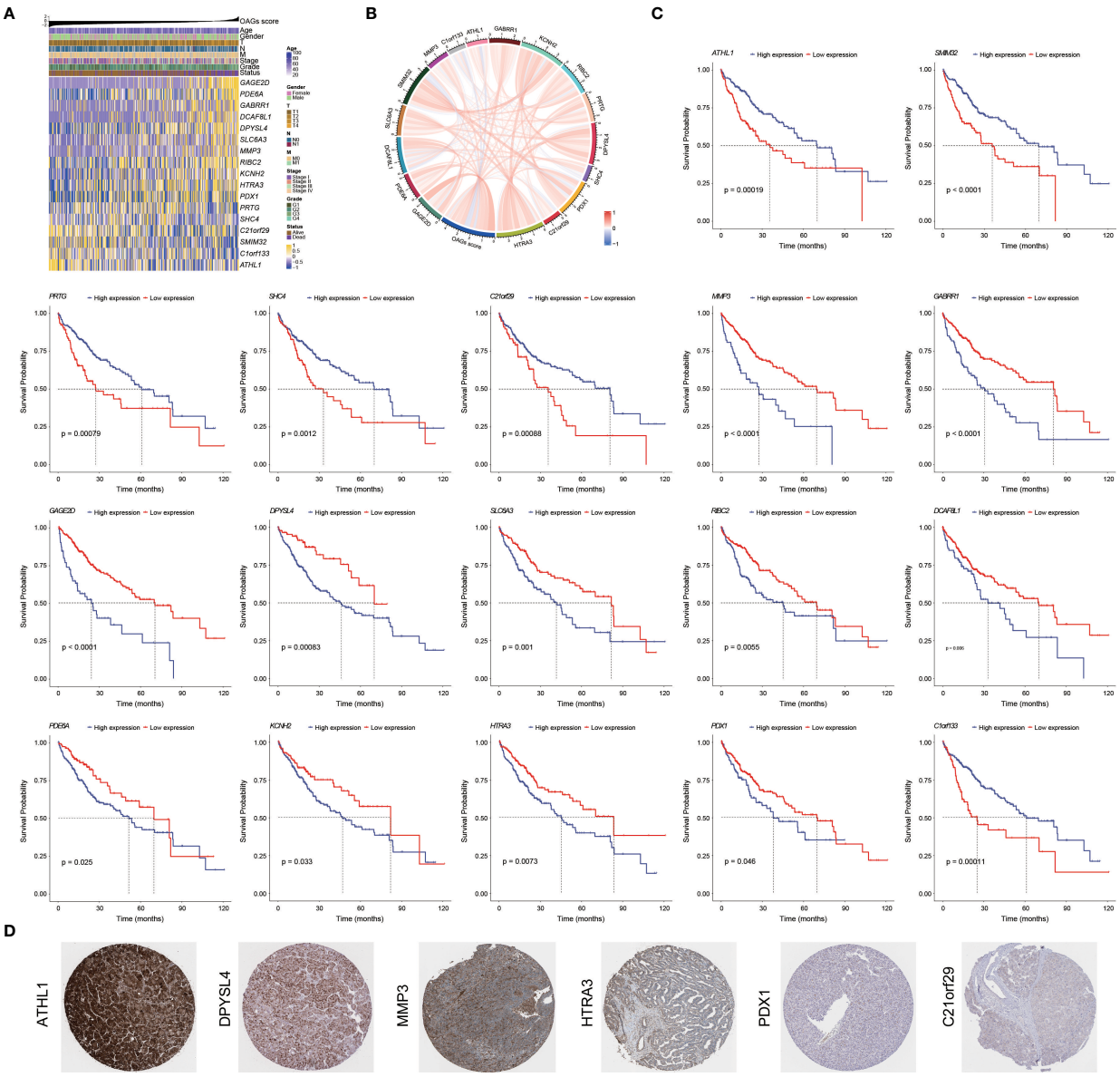

FIGURE 4
Prognostic significance and contribution of overweight-associated genes (OAGs) involved in the signature. **(A)** The heatmap for OAG expression profiling. **(B)** The correlation between OAG expression and OAG score. **(C)** Kaplan–Meier curve analysis based on the expression of single OAG. **(D)** The immunohistochemistry staining of OAG protein expression in TCGA-HCC samples.

and interestingly, significantly co-occurrence pairs were enriched in both groups except two special pairs (*CTNNB1-AXIN1* and *CTNNB1-TP53*) in the low OAG score group, demonstrating mutually exclusive alterations (Figure 6E). By further statistical analysis, it was highlighted that *TP53* (37.71% *vs.* 22.67%) and *DNAH10* (8.00% *vs.* 0.58%) were significantly more prevalent in the high OAG score group; but comparatively, none of the genes was significantly more altered in the low OAG score group instead (Figure 6F). Furthermore, *TP53* or *DNAH10* alterations were positively correlated with the OAG score (Figures 6G, H), and correspondingly, the *TP53* or *DNAH10* altered group had inferior OS indeed (Figures 6I, J). The in-depth investigation of specific altered locations did not recognize any difference between these two groups (Figures 6K, L).

TABLE 3 Univariate and multivariate Cox regression analyses for OAG score and clinical features in TCGA-HCC cohort.

Variable	Univariate			Multivariate		
	HR	95% CI	p-Value	HR	95% CI	p-Value
OAG score						
High/low	4.60	3.09–6.83	<0.01**	4.26	2.32–7.81	<0.01**
Age						
≥61/<61	1.28	0.90–1.80	0.17	2.13	1.18–3.85	<0.05*
Gender						
Male/female	0.81	0.57–1.15	0.23	1.59	0.83–3.03	0.16
Body mass index						
≥ 25/<25	0.80	0.56–1.17	0.25	0.88	0.50–1.53	0.64
Alcohol use						
Yes/no	1.08	0.75–1.57	0.68	0.53	0.25–1.14	0.10
Vascular invasion						
Yes/no	1.34	0.89–2.03	0.16	1.12	0.63–1.99	0.70
Grade						
High/low	1.11	0.78–1.60	0.56	1.73	0.98–3.06	0.06
T stage						
High/low	2.47	1.73–3.52	<0.01**	1.05	0.12–8.83	0.97
N stage						
N1/N0	1.19	0.17–8.60	0.86	1.17	0.39–5.18	0.32
M stage						
M1/M0	4.06	1.27–12.9	0.02*	2.63	0.71–9.69	0.15
Clinical stage						
High/low	2.38	1.64–3.45	<0.01**	1.80	0.22–14.6	0.58
Virus status						
Positive/negative	0.52	0.35–0.76	<0.01**	0.67	0.35–1.31	0.25

OAG, overweight/obesity-associated gene.
*: $p < 0.05$; **: $p < 0.01$.

3.8 TME characteristics associated with OAG score

Subsequently, TME was further evaluated between the high and low OAG score groups. Although there was no statistically significant difference in ESTIMATE score between these two groups, a higher OAG score indicated an increased TIDE ($p < 0.05$) score but lower tumor purity ($p < 0.01$, Figures 7A–C). Moreover, it was identified that indeed the expression of chemokines (*CCL7*, *CCL13*, *CCL20*, *CCL26*, *CXCL1*, *CXCL3*, *CXCL5*, and *CXCL6*), paired receptors (*CCR1*, *CCR3*, *CCR8*, *CCR10*, *CXCR2*, and *CXCR4*), and a large number of MHC molecules (*HLA-DQA*, *HLA-DOB*, *HLA-DQB1*, *HLA-DPA1*, *HLA-DMB*, *HLA-DRA*, *HLA-DMA*, *HLA-DOA*, *TAP1*, and *TAP2*) significantly elevated in the high OAG score group (Figure 7D). The expression of *CCL14*, *CCL15*, *CCL16*, *IL6R*, and *ICOSLG* was upregulated in the low OAG score group. Furthermore, the OAG score was also positively correlated with a majority of other immunomodulators. Notably, it was further found that there was almost no significant difference in the expression of effector genes of CD8+ T cells, NK cells, and Th1 cells, although several dendritic cell- and macrophage-associated effector genes, including *SLAMF8*, *LILRB4*, *IL21R*, *CLEC5A*, *C1QA*, *CSF1R*, *CYBB*, and *LILRA2*, were significantly upregulated in the high OAG score groups (Figure 7E). Correspondingly, cancer immunity cycle activity analysis

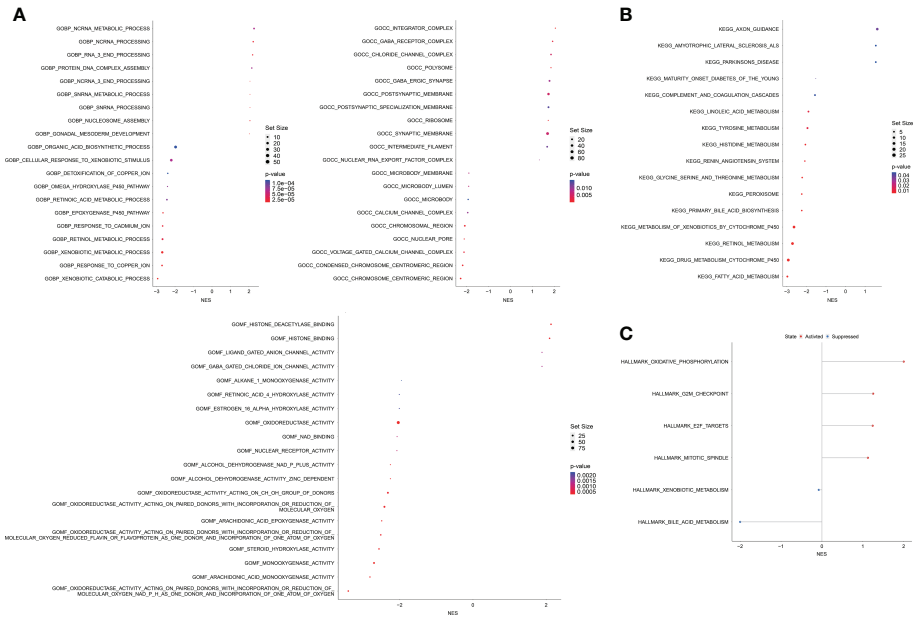

FIGURE 5
Biological enrichment analysis between high and low OAG score groups. **(A)** Gene ontology enrichment analysis. **(B)** Kyoto Encyclopedia of Genes and Genomes enrichment analysis. **(C)** Gene set enrichment analysis based on the Hallmark pathways. OAG, overweight/obesity-associated gene.

revealed that the release of cancer cell antigens (Step 1) and trafficking of immune infiltrating cells to tumor cells (Step 4: basophil recruitment, eosinophil recruitment, myeloid-derived suppressor cell (MDSC) recruitment, and neutrophil recruitment) were upregulated in the high OAG score group. In contrast, the activity of killing cancer cells (Step 7) was downregulated (Figure 7F). Lastly, it was found that the OAG score was positively correlated with a majority of the expression of immune checkpoint genes, especially *TIM3*, *CD80*, *LAIR1*, and *VTCN1* (Figure 7G). Moreover, there existed a close relationship in the expression between *PD-L1*, *PD-1*, *CTLA4*, *LAG3*, *TIM3*, *TIGIT*, *IDO1*, *CD80*, *LAIR1*, and *CD200R1*.

3.9 Underlying response of sorafenib, TACE, and chemotherapeutic treatments

Sorafenib remains the standard of care in the first-line treatments for HCC patients. In the present study, the relationship between the sorafenib responder and the OAG score was then investigated. Noticeably, it was discovered that responders to sorafenib had higher OAG scores compared with those without response ($p = 0.002$, Figure 8A). Regarding the role of each involved OAG, it was noticed that the lower expression of *ATHL1* but higher expression of *GABRR1*, *KCNH2*, *RIBC2*, *PDE6A*, and *PDX1* was significantly correlated with the response of sorafenib treatment in HCC ($p < 0.05$, Figure 8B). Conversely, response assessment for patients treated with TACE treatment showed that responders had markedly lower OAG scores than non-responders ($p < 0.001$, Figure 8C). At the same time, the expression of *ATHL1* and *C1orf133* was positively correlated with the response of TACE treatment in HCC ($p < 0.05$, Figure 8D). In addition, the GDSC database analysis further demonstrated that the predicted IC50 values of paclitaxel, vinblastine, vorinostat, vinorelbine, methotrexate, 5-FU, belinostat, and tivozanib were significantly lower in the high OAG score group ($p < 0.05$, Figure 8E), whereas the predicted IC50 values of erlotinib and phenformin were significantly lower in the low OAG score group ($p < 0.05$, Figure 8E). Overall, the OAG score was of guiding significance in treatment selection.

4 Discussions

HCC is a type of malignant cancer with extraordinary heterogeneity, usually accompanied by concomitant multiple molecular heterogeneities in genomic instability, transcriptomic disturbance, and signaling maladjustment. In most cases, HBV/HCV infections or alcohol-induced chronic hepatitis and fibrosis are thought as the major causes contributing to HCC. Nevertheless, the pandemic of overweight/obesity has gradually changed such a circumstance, and a growing body of evidence has demonstrated that

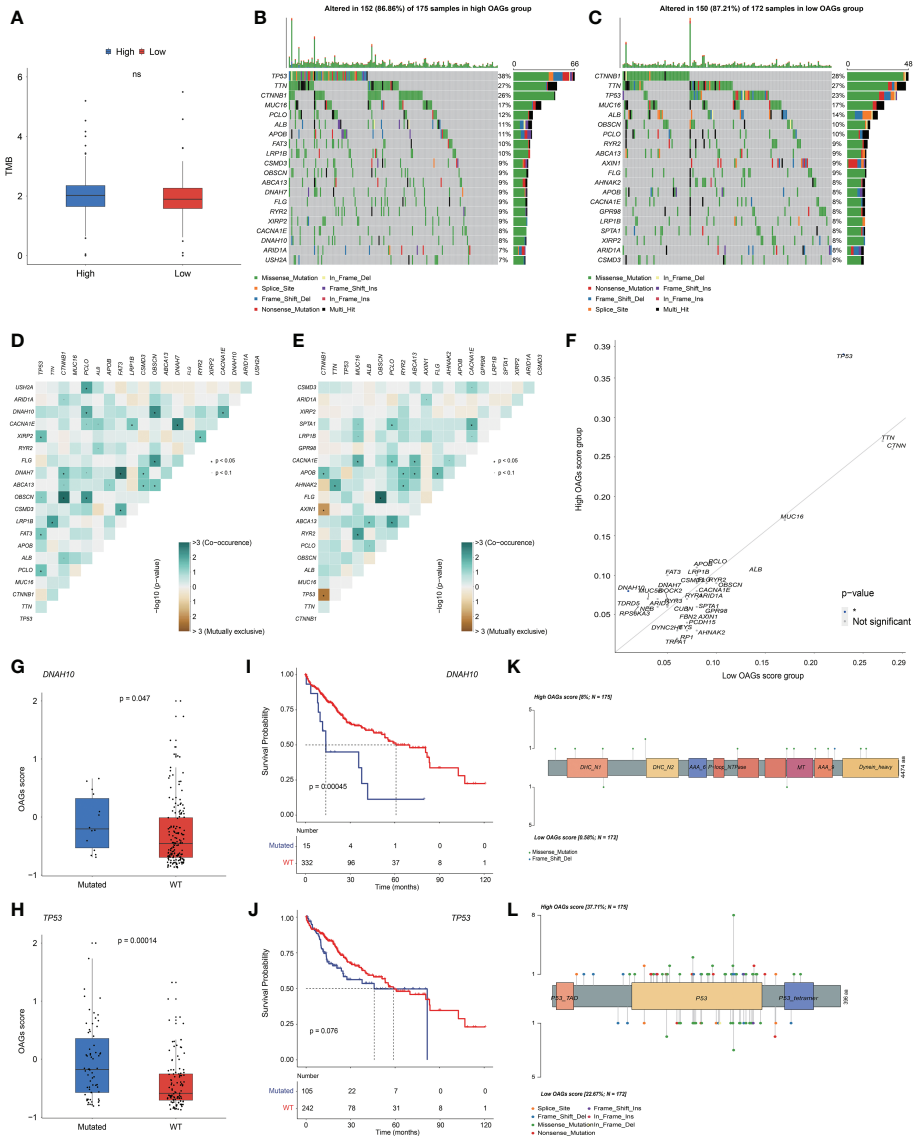

FIGURE 6
OAG score associated with distinct somatic genome. **(A)** The evaluation of TME level between high and low OAG score groups. **(B)** Oncoprint plot for genomic alterations of patients from high OAG score group. **(C)** Oncoprint plot for genomic alterations of patients from low OAG score group. **(D)** The heatmap of mutually co-occurrence and exclusive alterations of the top 20 altered genes in high OAG score group. **(E)** The heatmap of mutually co-occurrence and exclusive alterations of the top 20 altered genes in low OAG score group. **(F)** The somatic alteration enrichment analysis for high and low OAG score groups. **(G)** *DNAH10* somatic alteration associated with OAG score. **(H)** *TP53* somatic alteration associated with OAG score. **(I)** Kaplan–Meier curve analysis between patients with *DNAH10* somatic alterations or not. **(J)** Kaplan–Meier curve analysis between patients with *TP53* somatic alterations or not. **(K)** The profiling of alteration sites of *DNAH10* somatic alterations between high and low OAG score groups. **(L)** The profiling of alteration sites of *TP53* somatic alterations between high and low OAG score groups. OAG, overweight/obesity-associated gene; TME, tumor microenvironment.

overweight and obesity are highly correlated with increased risk and earlier recurrence in HCC (32, 33). Nevertheless, precise molecular mechanisms through which overweight/obesity promotes the development and progression and potentially affects the therapy response of HCC are scarcely known. As a multiplicative interaction between overweight/obesity and alcohol despite low and moderate alcohol intakes, over other risk factors, increases the risk and death due to HCC (34, 35), in the present study, a comprehensive overweight/obesity-associated transcriptome was identified after excluding HCC patients with the alcohol consumption history. Notably, overweight/obesity-associated transcriptome was found to be mainly involved in the metabolic processes, and this overweight/obesity-associated metabolic transcriptome was closely

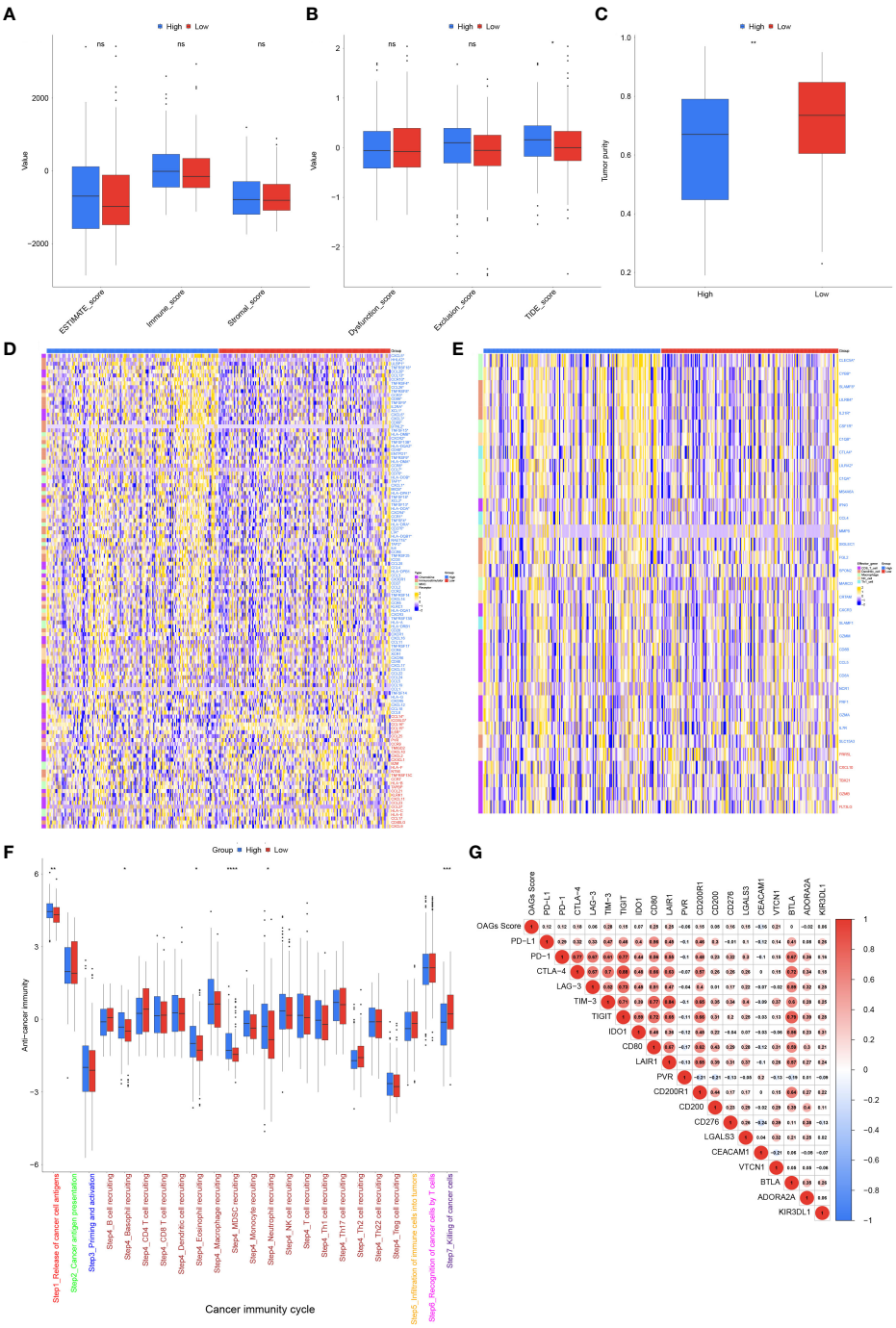

FIGURE 7
Tumor microenvironment (TME) associated with OAG score. **(A)** The stromal, immune, and ESTIMATE scores between high and low OAG score groups. **(B)** The dysfunction, exclusion, and TIDE score between high and low OAG score groups. **(C)** The evaluation of tumor purity. **(D)** Comparison of immunomodulator-related gene expression between high and low OAG score groups. **(E)** Transcriptomic profiling of effector genes of tumor-infiltrating immune cells in high and low OAG score groups. **(F)** Evaluation and comparison of anti-cancer immunity by cancer immunity cycle between high and low OAG score groups. **(G)** Correlation between OAG score and immune inhibitory checkpoint gene expression. OAG, overweight/obesity-associated gene.

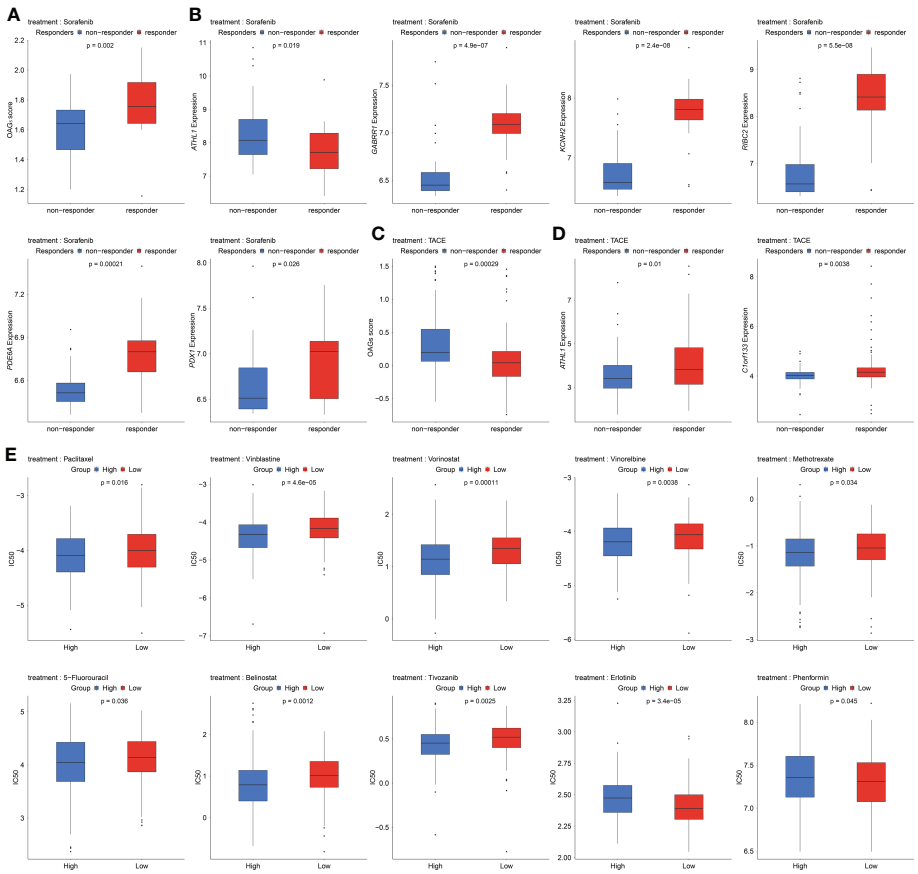

FIGURE 8
Underlying response of sorafenib, transcatheter arterial chemoembolization (TACE), and potential chemotherapeutic treatment regimens. **(A)** OAG score associated with sorafenib treatment response in GSE109211 dataset. **(B)** A part of OAG (*ATHL1*, *GABRR1*, *KCNH2*, *RIBC2*, *PDE6A*, and *PDX1*) expression also correlated with sorafenib treatment response in GSE109211 dataset. **(C)** Correlation between OAG score and TACE treatment response in GSE104580 dataset. **(D)** ATHL1 and C1orf133 expression correlated with TACE treatment response in GSE104580 dataset. **(E)** The GDSC database analysis revealed that OAG score could distinguish patients potentially sensitive to different chemotherapeutic regimens. OAG, overweight/obesity-associated gene; GDSC, Genomics of Drug Sensitivity in Cancer.

correlated with not only clinical outcome but also the immune microenvironment and immunomodulation in HCC. By the foundation of this, a more robust OAG signature was constructed, whereas clinical association analysis showed that the OAG signature was not correlated with BMI in the whole TCGA-HCC cohort. Regarding non-alcoholic HCC patients, a higher OAG score was associated with a higher proportion of individuals with overweight/obesity (51.01% *vs.* 41.10%), but there was no statistically significant difference either. In most cases, risk factors of viral infection, alcohol, smoking, overweight/obesity, and others did not occur alone in HCC, and usually, they were synergistic risk factors (36, 37). Therefore, multiplicative interaction between risk factors mainly caused clinical features of gender, age, BMI, and others, which were not independent prognostic factors; meantime, it was identified that there was nearly no positive correlation between the OAG signature and BMI. In addition, heterogeneity between different individuals also causes the deviation of BMI; unfortunately, there is a lack of systemic classification methods defining cases of overweight/obesity (38). In the present study, it was identified that the OAG signature was the only independent prognostic factor in three retrospective cohorts, and the OAG signature performed quite well in prognosis prediction for HCC patients, even for early-stage individuals. Moreover, the OAG score was highly correlated with molecular characteristics and the immune microenvironment and had the potential capacity of evaluating the response of sorafenib, TACE, or chemotherapy treatment.

Regarding the novel established the OAG signature, which contained a total of 17 genes and was independent of clinical features in HCC, within the OAG signature, 6 and 11 of these 17 OAGs, respectively, served as protective factors and risk factors at the transcriptomic level. Furthermore, enrichment analysis revealed that identified OAGs were majorly involved in the

metabolic processes. In contrast, it should be emphasized that the expression of only *DPYSL4*, *MMP3*, *HTRA3*, *PDX1*, *C21orf29*, and *ATHL1* proteins was ever observed in the cytoplasm/membrane by IHC staining analysis among HCC patients, of which the expression of *DPYSL4*, *MMP3*, and *ATHL1* proteins was clearly detected in all involved samples. As reported, *DPYSL4* was associated with glycolysis (39) and hypoxia (40) in HCC, and meanwhile, its overexpression was proved to be correlated with the progression and metastasis of HCC. *MMP3*, encoding a kind of protein as a member of matrix metalloproteinase, was well known to be involved in tumor progression and invasion (41), while specific peptide inhibitors targeting MMP3 could suppress HCC cell migration (42). In contrast, the function or role of *HTRA3*, *PDX1*, *C21orf29*, or *ATHL1* in HCC was still unknown, and it was the first time that this is revealed in the present study that their expression was significantly correlated with prognosis. Of note, it should be highlighted that *ATHL1* expression was correlated with prognosis and performed well in prognosis prediction. More impressively, downregulation and upregulation were significantly associated with sorafenib and TACE treatment response, respectively. *ATHL1*, encoding a protein-glucosyl-galactosyl-hydroxylysine glucosidase (PGGHG), was mainly involved in the carbohydrate metabolic process, and three carboxyl residues, Asp301, Glu430, and Glu574, were responsible for the functional role of PGGHG (43). Altogether, it could be inferred that inhibition of *ATHL1* expression or PGGHG activity before sorafenib treatment might improve the therapeutic response. In addition, the IHC staining of the expression of *GABRR1*, *GAGE2D*, *KCNH2*, *PRTG*, *SHC4*, and *SMIM32* proteins was unknown and not reported in the HPA database and, hence, needs further investigations at the protein level. In our study, the expression of *GABRR1*, *GAGE2D*, *KCNH2*, *PRTG*, *SHC4*, and *SMIM32* was significantly correlated with OS of HCC, and the expression of *GABRR1* and *KCNH2* was associated with sorafenib treatment response. *C1orf133* was known as a kind of ncRNA *SERTAD4-AS1*, and also its expression was first identified in our study to be correlated with prognosis of HCC and even associated with TACE treatment response.

Dysregulation of hepatic metabolisms, such as oxidative phosphorylation, glycolysis, and fatty acid metabolism, was critical to the development and progression of liver disease, especially in patients with non-alcoholic hepatitis disease (44, 45). Similarly, GO and KEGG pathway enrichment disclosed that the aberrantly regulated biological activities associated with the OAG score in the present study were abundantly enriched with genes involved in the cytochrome P450-mediated metabolism, fatty acid metabolism, amino acid metabolism, retinol metabolism, and xenobiotic metabolism. Cytochrome P450-mediated metabolism usually caused the accumulative reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radical, which played a key role in contributing to steatohepatitis (46) and promoting invasiveness of HCC cells (47). The dysregulation of fatty acid metabolism might directly result in the anomalous activities of peroxisome proliferation-activated receptors (PPARs: α, β, γ) and related signaling pathways, which acted as fatty acid sensors (48). Moreover, these PPAR members were critical transcription factors regulating mitochondrial functions and energy homeostasis (49); thus, some pharmacological strategies of PPAR agonists have emerged and are associated with improved clinical outcomes (50). Moreover, perturbation of amino acid metabolism was also correlated with the progression of hepatic live diseases (51). Noticeably, cepharanthine treatment could inhibit HCC cell proliferation and migration by regulating amino acid metabolism (52). Of note, hepatic tissue in individuals stores almost 70% of retinoids (53); as reported, the inhibition of retinoids or the loss of hepatic retinoid signaling potentially leads to oxidative stress (54), which was associated with the progression of liver diseases (55). Moreover, retinoids were involved in many biological activities, including apoptosis promotion and inflammation response; altogether, retinol metabolism was markedly correlated with the development and progression of HCC (56). In addition, Hallmark pathways of oxidative phosphorylation and cell cycle/DNA replication-related signaling were abundantly enriched in the HCC patient group with inferior survival further confirming that increased oxidative stress/oxidative phosphorylation significantly promoted the progression of HCC (57). Furthermore, oxidative phosphorylation activation was also correlated with chemotherapeutic resistance (58). Overall, dysregulated metabolisms associated with the OAG score enormously affected clinical outcomes and immunomodulation or inflammatory regulation in HCC.

From another aspect, genomic characterization can offer a compelling framework to demonstrate the functional significance and discover key genes stimulating the development and progression of HCC. Nevertheless, evidence is mounting that more and more therapeutic regimens targeting on-oncogene alterations are engendered, compared to the tumor suppressor genes or recurrently altered passenger genes (59). However, there were only a few disparities in the genomic characterizations between the high and low OAG score groups. Despite that *TP53* and *DNAH10* frequently altered in the high OAG score groups, no specific alteration sites of *TP53* or *DNAH10* were significantly more prevalent. According to the Catalogue of Somatic Mutations in Cancer database, over 30% of all HCC patients harbored at least one alteration in *TP53*, ranking first in terms of alteration frequency in HCC. As a tumor suppressor gene, *TP53* alterations were expectedly correlated with the development of progression of HCC (60), and consistent with this, HCC patients with high OAG scores had more altered *TP53* and inferior OS. Generally, cells with altered TP53 protein could escape from apoptosis and gradually

develop into HCC cells due to DNA damage events, which could also contribute to HCC progression (61). Moreover, *DNAH10* alteration was positively correlated with the OAG score, and it was found that patients harboring *DNAH10* alterations had significantly worse OS in HCC, compared with wild-type patients. *DNAH10*, namely, dynein axonemal heavy chain 10, encodes a protein of inner arm dynein heavy chain (62); however, the role of *DNAH10* in liver tissue is scarcely known. In contrast, there were several studies revealing that altered *DNAH10* was correlated with the elevated level of high-density lipoprotein cholesterol (63), adipocyte function (64), and adipocyte differentiation (65). Based on the experiment of RNAi-knockdowns for *DNAH1* expression in *Drosophila*, the total triglyceride levels were elevated within the body (66). Altogether, it could be implied that altered *DNAH10* might aggravate the progression of HCC by influencing lipid metabolism, which needed further experimental validation. Interestingly, there existed two cases of *CTNNB1-AXIN1* and *CTNNB1-TP53* exhibiting mutually exclusive alterations in the low OAG score group, suggesting that their effects in the same pathway were probably redundant and that there was an epistatic association between these two genes; however, this phenomenon did not occur in the high OAG score group.

Multi-kinase inhibitors, such as sorafenib and lenvatinib, are still the first-line treatment, while immune checkpoint blockades, alone or in combination with other regimens, have revolutionized the clinical management and treatment of HCC (67). Nevertheless, the molecular mechanisms influencing immune response and evasion in HCC remain to be fully elucidated. Impressively, it was initially identified that overweight/obesity-associated transcriptome in the present study was markedly associated with immunomodulation and the immune microenvironment of HCC. Moreover, most chemokines, receptors, immunomodulators, and MHC molecules were upregulated in the high OAG score group, implying that a high OAG score potentially had higher activity in antigen presentation and processing as well as the promoting recruitment of antigen-presenting cells, CD8+ T cells, and Th17 cells. Comparatively, the cancer immunity cycle was a more comprehensive reflection of the immunomodulation system, representing the immune response to tumors (21). Controversially, the activity of killing cancer cells (Step 7) was downregulated in the high OAG score group, which presented with a higher level of inflamed TME and increasing activity in both the releasing of cancer cell antigens (Step 1) and part of the trafficking of immune infiltrating cells to tumor cells (Step 4). This discordance might be due to the positive association between the OAG score and PD-L1/PD-1 expression as well as a majority of immune checkpoint gene expression, indicating that these immune checkpoints would suppress cancer immunity and lead to immune evasion (68). In addition, the high OAG score group had a higher level of TIDE score, which has been proven to be negatively correlated with the infiltration of effective CD8+ T cells within tumors (19). Altogether, it was reasonably believed that the final activity of anti-cancer immunity might be downregulated in the high OAG score group. In summary, we strongly recommended that immunosuppressive factors should be inhibited first to prevent the exclusion of T cells from infiltrating tumors (69), which could improve the response of immunotherapy in the high OAG score group. However, immunotherapy was probably not suitable for HCC patients with a low OAG score because of a low level of inflamed TME and immune checkpoint gene expression.

Reversely, over-inflammation in the high OAG score group could substantially stimulate the progression of HCC, while targeting inflammation could become a promising treatment strategy for these patients (70). Sorafenib, having been approved by Food and Drug Administration as the standard treatment for HCC (71), could inhibit inflammatory pathways and reduce liver fibrosis in cirrhotic rats (72). Consistently in the present study, a higher OAG score was significantly correlated with the response to sorafenib treatment, probably owing to the higher level of inflamed TME among these patients. As exhibited, Macrophage M0 was abundantly enriched in the high OAG score group. Compared to the single drug sorafenib for HCC patients, depletion of macrophages by zoledronic acid or clodrolip in combination with sorafenib resulted in the stronger inhibition of HCC progression, angiogenesis, and even lung metastasis (73). Therefore, a combination treatment of sorafenib and zoledronic acid or clodrolip seemed to be more effective for patients with a high OAG score. In addition, it was further identified that the OAG score was negatively correlated with the response to TACE treatment. Regarding the relatively early-stage HCC patients, as suggested, patients with a lower OAG score associated with a lower level of inflamed TME are likely to receive the TACE treatment. Overall, it was demonstrated that the OAG score also had the potential to become a reliable and robust predictor for the response of sorafenib or TACE treatment, which would greatly help promote clinical management and precision medicine for HCC patients. The GDSC data analysis revealed that patients in the high OAG score group were likely to have a higher sensitivity to chemotherapy *via* the drugs paclitaxel, vinblastine, vorinostat, vinorelbine, methotrexate, 5-FU, belinostat, and tivozanib, whereas those in the low OAG score group seemed to be more sensitive to erlotinib and phenformin. However, it needed to be proposed that the evaluation of chemotherapeutic sensitivity was mainly based on pharmacogenomic analysis in cancer cells (74), so further investigations in animal models or clinical trials are needed for verification.

The comprehensive overweight/obesity-associated metabolic transcriptome was profoundly correlated with clinical outcome, immunomodulation, and the immune microenvironment, and afterward, the novel constructed OAG signature could function as an effective independent predictor of prognosis and determine the molecular characterization and TME of HCC, as well as predict the response of sorafenib and TACE treatment. In contrast, there still existed some limitations that should be noted. First, this study was mainly based on the public database and more likely as a retrospective cohort analysis; thus, prospective studies are needed for validation. Second, it was powerful to use machine learning for the construction of the OAG signature, but the bioinformatics analysis still predominated in this process, and it might hinder the clinical significance of some overweight/obesity-associated genes in HCC. Because of this, we found that the OAG score was not significantly associated with BMI; thus, the OAG signature might lack the power to distinguish HCC patients from overweight/obesity patients. However, an in-depth investigation of overweight/obesity-associated transcriptome provided more information about molecular characteristics, the immune microenvironment, and therapy response. Finally, we indirectly evaluated the underlying response of immunotherapy, and HCC patients treated with immunotherapy were not really verified in our study, so more clinical trials should be designated for further exploration. Overall, it might be concluded that transcriptomic characterization driven by overweight/obesity (or higher BMI) played a vital role in the progression of HCC meanwhile, which was also highly associated with the immune microenvironment and therapy response.

5 Conclusions

The findings in the present study first disclosed that comprehensive overweight/obesity-associated metabolic transcriptome was significantly correlated with prognosis and TME of HCC, and a novel constructed OAG signature exhibited better performance in prognosis prediction. Moreover, the OAG signature was also associated with the response of sorafenib, TACE, or chemotherapy. This study could offer a clinically applied tool to promote the management of HCC and increase the need for a clear strategy of precision medicine in HCC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

N-NF and B-MY proposed and designated this work. N-NF and X-YD collected and processed the raw data. N-NF, X-YD and Y-SZ conducted the bioinformatics analysis. Y-SZ, Z-KJ and X-HW prepared and made visualizations and were responsible for figures and tables. N-NF and X-YD wrote the original manuscript. N-NF and B-MY revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1061091/full#supplementary-material

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Obesity and myeloma: Clinical and mechanistic contributions to disease progression

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Obesity and obesogenic behaviors are positively associated with both monoclonal gammopathy of unknown significance (MGUS) and multiple myeloma (MM). As the only known modifiable risk factor, this association has emerged as a new potential target for MM prevention, but little is known about the mechanistic relationship of body weight with MM progression. Here we summarize epidemiological correlations between weight, body composition, and the various stages of myeloma disease progression and treatments, as well as the current understanding of the molecular contributions of obesity-induced changes in myeloma cell phenotype and signaling. Finally, we outline groundwork for the future characterization of the relationship between body weight patterns, the bone marrow microenvironment, and MM pathogenesis in animal models, which have the potential to impact our understanding of disease pathogenesis and inform MM prevention messages.

KEYWORDS

obesity, multiple myeloma, diet, treatment, hematological malignancies

1 Introduction

Multiple Myeloma (MM) is a fatal plasma cell dyscrasia characterized by bone marrow (BM) infiltration and lytic bone lesions. MM is the second most common hematologic malignancy, with approximately 34,460 new cases diagnosed in 2022 in the USA alone (1). Advancements in MM treatments have dramatically improved the median survival time in patients over the past two decades; however, MM remains incurable. Existing evidence points to obesity or obesogenic behaviors as plausible targets for MM prevention. Obesity remains one of the few established risk factors for both MM and its premalignant precursor states of monoclonal gammopathy of unknown significance (MGUS) and smoldering MM (SMM), and is the only known modifiable risk factor. For individuals at risk for MM, further understanding of the relationship between disease risk and body weight are urgently needed.

Obesity, defined as a body mass index (BMI) ≥30, affects approximately 42% of adults in the US (≥18 years of age), and has been rising concurrently with the prevalence of cancer over the past two decades (2). Obesity-related conditions (eg. heart disease, stroke, type 2 diabetes and

certain types of cancer) led to medical costs of ~$173 billion in 2019, and these conditions are among the leading causes of preventable, premature death in the US (2). Extremely high BMI has been associated with an increased risk of at least 13 cancers, including MM (3–6), and many studies have demonstrated a significant reduction in cancer risk with weight loss in women (7). Adipose tissue, commonly known for its role in energy storage, commonly increases with weight gain and increased BMI, and represents an endocrine organ that secretes bioactive compounds. It is composed of adipocytes, macrophages, lymphocytes, preadipocytes, fibroblasts, and endothelial cells. Adipose tissue composition depends on type, location, age, and degree of obesity, among other factors. Two types of adipose tissue most well studied are white adipose tissue (WAT) and brown adipose tissue (BAT) (8), but recent work has begun to characterize other adipose depots such as bone marrow adipose tissue (BMAT) (9), beige adipocytes (10, 11) and perivascular adipose tissue (PVAT) (12). The contributions of these depots to obesity/metabolic disease, and their responses to obesity, may suggest new anti-obesity treatments, or further illuminate how obesity initiates such devastating diseases. Specific to MM, most research currently investigates contributions of WAT or BMAT to MM, since the BM is the primary location of myeloma cell growth, and since both WAT and BMAT increase in obesity (13). As myeloma cells are known to interact closely with neighboring cells, their interaction with BM adipocytes represents an interesting new field of research, especially since BMAT also increases with age (14), another major risk factor for MM.

Some studies have attempted to understand the link between increased MM risk and obesity through biological mechanisms, as described below. Adipokines, such as leptin and IL-6, induce myeloma cell survival and proliferation, but targeting adipokines in MM or cancer generally has not had great translational success and new targets, based on a better understanding of the pathology, are needed. Very few studies specifically interrogate the mechanistic links between obesity and myeloma disease progression, perhaps due to technical challenges of preclinical models, and thus our overall understanding of the effects of obesity on MM and its microenvironment, is still nebulous. The aim of this review is to provide a summary of the current epidemiologic evidence associating obesity with myeloma incidence, the status of the field attempting to connect cellular mechanisms to this increased risk, and our recommendations for preclinical (animal) studies to address some of the remaining gaps in this field.

2 Obesity, diabetes, and multiple myeloma

2.1 Obesity and risk for MM incidence

Obesity has long been considered a major risk factor for cancer and is the only known modifiable risk factor for multiple myeloma. At every stage of the disease progression, obesity plays a part, but the greatest impact may happen before a diagnosis is made, in the precursor disease state. Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant condition defined by the presence of a monoclonal paraprotein in the blood, a proliferation of clonal plasma cells in the BM (less than 10%), and the absence of end organ damage (15). MGUS occurs in 3.2% of persons aged 50 and older and 5.3% of those over age 70 (16), and it has a persistent risk of progression to MM of about ~1% per year (17). A recent systemic review found that increased BMI and obesity are implicated in MGUS development, as well as the progression to overt MM (18). Many other studies agree: the International Agency for Research into Cancer (IARC) and the World Cancer Research Fund cite greater body fatness as a contributor to the risk for MM development (5). The risk ratio (RR) of myeloma incidence has been found to be significantly increased (RR=1.11, p<0.0001) in both women and men, analyzed separately, based on a meta-analysis of 6 or 7 studies, respectively (per 5 kg/m^2 increase in body weight) in a 2008 study (18). A retrospective study of PET/CT data found that patients who were recently diagnosed with MM had higher abdominal fat cross sectional area and higher fat metabolic activity compared to patients with MGUS (19).

The Age Gene/Environment Susceptibility-Reykjavik Study (AGES-RS) has since revealed that a high midlife BMI is associated with increased risk of progression from MGUS to MM and other lymphoproliferative diseases (20). Similarly, a retrospective study from the US Veterans Health Administration in 2017 showed weight status and obesity are both associated with increased risk of transformation of MGUS to MM (21). Most recently, a retrospective analysis in 2022 within the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial revealed a 35% increase in odds of progression from non-IgM MGUS to MM with each 5 kg/m^2 increase in BMI (22).

In 2018, data gathered from the Nurses' Health Study (NHS), Health Professionals Follow-Up Study (HPFS), and Women's Health Study (WHS), revealed a positive association of both cumulative average adult BMI and young adult BMI with MM risk (23). MM incidence was positively associated with adult BMI, with relative risks of 1.2 for overweight, 1.2 for class 1 obesity, and 1.5 for class 2 or 3 obesity (6). Over a twenty-year observational period, MM risk is increased with increased hip circumference and medium-increase body trajectory compared to lean-stable body trajectory. Interestingly, extreme weight cycling (with net weight gain and at least one episode of 20 pound or greater weight loss) was recently associated with increased risk for MM compared to maintained weight (24).

2.2 Obesity is a controversial factor in multiple myeloma progression and MM-induced mortality

A prospective study on the influence of excess body weight and risk of death from cancer found current patterns of obesity in the United States could account for 14% of all deaths from cancer in men and 20% in women (4). The relative risk of death from cancer for men was 1.52 and 1.62 for women, with BMI significantly associated with death due to MM specifically (4). Pooled analyses of prospective trials from 2011 and 2014 revealed a 9-15% and 52-54% increased risk of MM mortality for overweight (BMI ≥25) and obese individuals, respectively (25, 26). In African American (AA) populations, mortality due to MM increases monotonically with BMI increase, with hazard ratios up to 1.43 for BMIs of 35 kg/m^2 or greater, using data from seven prospective cohorts tracking mortality among 239,597 adults in the AA BMI-Mortality Pooling Project (27).

Still, not all studies agree, and a recent study of 563 subjects showed BMI before (for both sexes) and at the time of MM diagnosis

(for males) are not associated with overall survival in MM, and, surprisingly, that a higher BMI at diagnosis is associated with *better* overall survival for females (28). Similarly, one abstract presented at the 16th International Myeloma Workshop in 2017 by a team from the Mayo Clinic found that, in patients with heavily pretreated MM, obesity (BMI >30) was associated with better outcomes compared to those who were not obese (BMI <30) (29). Interestingly, although the response rates for these groups to treatments were similar for the obese and non-obese groups, the progression free survival (PFS) and overall survival (OS) were significantly better in the obese group (29). The authors explain the that exact cause of this is unknown, but hope that future work will both confirm their findings and explore the mechanisms that physiological consequences of obesity may have on disease biology or drug metabolism (29).

Similarly, in a study of 1,087 MM patients, among those who received melphalan and total body irradiation (TBI), obese and severely obese patients had superior PFS and OS than did normal and overweight patients: PFS at 5 years was 23% in normal weight patients, 17% in overweight patients, 43% in obese patients, and 55% in severely obese patients (p=0.005) (30). This was not true for MM patients who received melphalan alone, suggesting that differences in how irradiation affects MM in obese vs lean individuals may drive the differences in patient responses (30). Overall, some studies support an associate between increased BMI or obesity, and increased mortality in MM, while other studies suggest that obesity can, surprisingly, have the opposite effect on MM patient outcomes. Thus, more research into obesity and response to MM treatments is needed and more work should be done to determine if weight loss strategies (diet, exercise, or drugs) are useful interventions in MM.

The controversial finding that obesity, a major risk factor for many cancers, can be protective in terms of cancer patient survival has been observed in many other types of cancer as well, and is termed the "obesity paradox" (31). As reviewed by Arnold et al., there are perhaps biological explanations for this, such as protection from cachexia [which occurs in ~30% of MM or lymphoma patients during treatment and increases mortality risk (32)], or advantages of increased body weight during aging. However, there are also a many plausible methodological reasons for the "obesity paradox", such as residual confounding, reverse causality, and a selection bias known as "collider bias" (31). In fact, blocking browning in white adipose seems to be an effective way to protect from cachexia-induced weight loss and muscle wasting, and thus it is likely not the total amount of adipose, but it's metabolic state, that determines its role in cachexia (33, 34). Furthermore, cachexia, a metabolic syndrome that can be present even in the absence of weight loss, can in fact frequently be obscured by obesity, leading to under-diagnosis and excess mortality (35). Thus, we should not interpret the observations about obesity and survival in MM, or any cancer, to mean that high BMI will reduce the risk of death or cachexia for cancer patients, or that gaining weight could be beneficial (31).

2.3 The diabetes- MM connection

Many publications of epidemiological data have found or suggested that type 2 diabetes mellitus (T2DM) is a risk factor for developing MM or causing worse clinical outcomes (36). For example, recent work from Shah et al. found that patients with MGUS, MM, amyloidosis, as well as some other lymphoproliferative disorders (LPDs), were more likely to have a preceding diabetes mellitus (DM) diagnosis compared to matched controls (37). However, the story is complicated, as they found that patients with DM were no more likely to progress from MGUS to MM, WM, amyloidosis, or other LPDs than non-diabetic patients. However, they note that they could not control for the use of anti-diabetic drugs that may lower rates of MGUS progression (37). A recent systematic review and meta-analysis analyzed 13 studies and concluded that T2DM does not increase risk of MM (34), but again the authors hypothesize that the use of hypoglycemic drugs, such as metformin, could explain why these patients do not have an increased risk of MM (34). Another recent study from Japan of 131,701 cancer patients, 6,135 of which had coexisting diabetes, found, perhaps not surprisingly, that survival was better for cancer patients without (versus with) diabetes (38). More interestingly, patients who had diabetes also had a higher risk of developing second primary cancer, specifically of MM, as well as uterine, liver, and pancreatic cancer (38). The presence of diabetes was identified from the prescription records of antidiabetic drugs, which included an array of drugs (metformin, insulin and insulin analogs (pen-type injection device), glucagon-like peptide 1 receptor agonists, dipeptidyl peptidase-4 inhibitors, sodium–glucose cotransporter 2 inhibitors, thiazolidines, glinides, alpha-glucosidase inhibitors and sulfonylureas) (38). Thus, even for patients on anti-diabetic drugs, diabetes was a risk factor for MM development (as a secondary cancer) (38). More on diabetes and MM specifically can be found in the reviews by Tentolouris et al. (38) and Fais et al. (39).

3 Treatment options for targeting obesity-related contributions to MM

The association between obesity and MM prompts a compelling argument for the use of metformin as a pharmacotherapy in patients with MGUS to reduce body weight, metabolic disease, or adiposity parameters, including BMI and waist circumference (40). Relatedly, metformin, a drug commonly used in diabetic patients, has promising anti-MM effects based on epidemiological data. Data from 2017 show a significant reduction in myeloma risk for patients with MGUS and cumulative metformin exposure >2 years and adjusted for the serum glucose level (41). Likewise, a cohort of US veterans with comorbid MGUS and diabetes mellitus (DM) treated with metformin displayed reduction in myeloma risk with metformin exposure >4 years (21). Similarly, in a recently study of 739,553 patients from Taiwan's National Health Insurance database, T2DM patients who were prescribed metformin within the first year of their diagnosis with T2DM had a lower risk of developing MM compared to T2DM patients who were not prescribed metformin (42).

Metformin also exhibits anti-myeloma effects *in vitro* and immunocompromised xenograft models (43), however this has not been tested in a diet-induced obesity (DIO) model. A recent study on *in vitro* and *in vivo* MM models demonstrates decreased proliferation of dexamethasone-resistant and -sensitive MM cell lines treated with metformin, with cell cycle assays showing arrest in the G0/G1 phase of MM.1S and H929 cells and arrest in the G2/M phase of RPMI8226 cells (44). Mouse survival was prolonged with metformin treatment,

with decreased U266 and H929 growth in BM (44). Moreover, metformin can reverse some of the negative effects on bone cause by DIO in mice (45), and has been shown to be beneficial to bone in other *in vivo* settings (46), which could make it useful in slowing osteolytic disease in MM. However, not all data on metformin concur; a recent study found mice pretreated with metformin for 4 weeks prior to inoculation of 5TGM1 MM cells had increased tumor burden, associated with increased osteolytic bone lesions and elevated osteopontin (OPN) expression in the bone marrow (47). *In vitro*, metformin increased MM cell attachment to osteoblasts, and increased OPN expression in preosteoblasts. This unexpected indirect pro-tumorigenic effect of metformin highlights the importance of fully elucidating the effects of metformin before using it as a treatment in MM (47). The implications of whether metformin could be repurposed in either MGUS as a preventative measure or in myeloma patients requires further investigation, as do other metabolically-focused therapies (40). A clinical trial is ongoing to assess whether metformin could be used in the future to help prevent MGUS or smoldering MM patients from progressing to MM (https://clinicaltrials.gov/ct2/show/NCT04850846).

Statins, or HMG-CoA reductase inhibitors, are another class of lipid-lowering medications that hold promise in MM (48). An analysis of 5,922 patients diagnosed with MM within the study period, the use of statins was associated with 21% reduction in risk of death among all patients, and of only those patients treated with novel agents (n=3,603), statins reduced mortality by 10% (48).

There is also promise in lowering MM risk through lifestyle changes promoting lower BMI. Data from the Women's Health Initiative Observational Study (WHI-OS) demonstrates significantly lower obesity-related cancer risk in women with intentional weight loss (greater than 5%) over three years compared to women with stable weight. These results are independent of race and/or ethnicity, baseline BMI, smoking status, or prior hormone use, illustrating the modifiable nature of obesity as a risk factor (7). Likewise, a retrospective study from 2008-2017 revealed decreased incidence of obesity-related cancer in patients with comorbid nonalcoholic fatty liver disease (NAFLD) and obesity who underwent bariatric surgery compared to patients with NAFLD and obesity who did not (49). Furthermore, high leisure-time physical activity is associated with a 7% lower risk of obesity-related cancer compared to low physical activity, and when combined with a BMI <25, the relative risk reduction is 27% for MM (50). Bariatric surgery would likely be beneficial for patients with morbid obesity, as lower overall cancer risk has been seen in obese women within the first 5 years after bariatric surgery (51), and based on the growing body of evidence to support the role of obesity as a dynamic influence on MM incidence. However, the fact that bariatric surgery, such as vertical sleeve gastrectomy and Roux-en-Y gastric bypass, cause bone loss (decreased bone mineral density), increased bone turnover, and increased risk of fracture cannot be overlooked for MM patients who already face bone loss and high fracture risk (52, 53). Overall, evidence for weight loss as an effective treatment or prevention method, especially when weighed against effects on bone loss, in MM is needed.

A greater understanding of how obesity contributes to drug resistance in MM is needed, as patients commonly relapse after therapies becomes ineffectual. The development of relapsed/refractory MM is the most common cause of MM death. The treatment regimen for newly-diagnosed MM patients depends on their risk as determined by genetic mutations within the myeloma cells themselves, as well as the patients' ability to tolerate autologous stem cell transplantation (ASCT), access to care, and other factors. In transplant eligible patients, frontline therapy typically includes bortezomib (or other proteasome inhibitors), lenalidomide (or other IMiDs [immunomodulatory drugs]), and dexamethasone (an anti-inflammatory, anti-myeloma steroid) for 3-4 cycles prior to ASCT. Patients who are not eligible for ASCT often receive similar treatments, but for 8-12 cycles (54). High-risk patients also may receive daratumumab (54), which binds to CD38, a cell surface marker overexpressed in myeloma cells, to recruit immune cells that target and kill MM cells. Maintenance therapy for patients includes lenalidomide, while bortezomib is added to this in the case of high risk patients. Many other therapies, such as melphalan (a chemotherapy) and newer, more targeted therapies are also used for front-line or maintenance therapy, or explored in the context of a clinical trial. How obesity affects the efficacy or long-term outcomes of these treatments, some of which (eg. dexamethasone) can lead to weight gain, remains a challenge in the field of precision medicine. Omega-3 fatty acids have been shown to enhance the killing effects of bortezomib and induce apoptosis in MM cell lines, but *in vivo* and clinical data are lacking (55). Overall, a better understanding of the effects of obesity on efficacy of current therapies could hold the potential to better tailor treatments to individual patients.

4 Biologic mechanisms linking obesity with MM

4.1 *In vivo* models

As with all cancer research, murine models of MM are a critical tool to understand the pathogenesis of the disease and in the development of novel therapeutic strategies. No model perfectly recapitulates the human tumor/tumor microenvironment, but many exist for MM and the best one(s) based on the hypothesis being tested should be identified. The choice of the system involves weighing the pros and cons of different model attributes, like the requirement of an intact host immune system, the use of human cell lines, and the host microenvironment. The inoculation of the myeloma cells can also be achieved with different methods such as tail vein injection to induce systemic disease, subcutaneous injection to induce solitary plasmacytomas, or intratibial injections to induce lytic bone lesions in the limb inoculated (56).

Many models simply do not show any effect of high fat diet (HFD) or obesity on MM disease progression, for various reasons. For example, many common immunodeficient strains (eg. severe combined immunodeficiency (SCID), non-obese diabetic/severe combined immunodeficiency (NOD/SCID), and NOD/SCIDIL2Rγ (NSG) mice), used for xenograft MM models, seem to be resistant to developing HFD-induced metabolic syndrome, and often do not become obese, due to a lack of adaptive immunity and defective innate immunity (55). In our lab, we found no significant differences in either HFD or weight cycling (high fat and low fat cycled dieting) in terms of survival compared to control diet, in a male SCID-beige MM.1S xenograft model (57).

However, some models can be used to investigate how obesity contributes to MM. In a 2015 study, Lwin et al., described the first DIO model useful to examine effects of obesity on MM development *in vivo*

(58). C57BL/6 mice, which are not normally permissive hosts for 5TGM1 murine myeloma cells, were placed on HFD (42% fat) or control diet (CD) for 5 weeks (Figure 1A). A significant increase in body fat and body weight were observed among the HFD mice. The mice were then inoculated with 5TGM1 myeloma cells *via* tail vein and their diet was maintained during the experiment. C57BL/6 mice on HFD developed features of MM including a significant increase in myeloma specific $IgG2_{bk}$ paraprotein and an accumulation of GFP-positive myeloma cells in the BM and spleen, while the mice on CD did not exhibit these characteristics. MicroCT analysis of the tibial trabecular bone volume (TBV) also showed significant bone loss in the myeloma-bearing mice on HFD (58). In this same study, myeloma permissive C57BL/KaLwRij mice were placed on HFD for 5 weeks before inoculation with 5TGM1 cells (tail vein), which resulted in tumor growth and osteolytic bone disease (58). Despite an increase in body fat due to the HFD, no significant differences in serum paraprotein, tumor burden within the BM, or TBV were observed. This indicates that the obese host environment created by HFD may not be directly promoting tumor growth or survival, but may be creating a myeloma-permissive microenvironment. Also in that manuscript, mice with a mutation in the leptin gene (*ob/ob*) resulting in biologically inactive leptin, were used to analyze myeloma development in a genetic model of obesity. 5TGM1 myeloma cells were inoculated into 12-week-old *ob/ob* mice *via* tail vein injection. No increase in serum paraprotein was observed and no GFP positive myeloma cells were detected in the BM or the spleen, perhaps due in part to the fact that leptin itself is a pro-myeloma adipokine and thus pro-tumor effects of obesity may be negated by the anti-tumor effects of leptin removal in these mice (61). This shows that there are distinct differences created by diet-induced obesity and leptin deficiency-induced obesity in terms of effects on tumors (58).

To investigate these differences between the models further, serum concentrations in DKK1 and adiponectin, as well as IL-6 and insulin growth factor (IGF)-1, which are connected to both obesity and myeloma, were measured (58). HFD was found to have no significant impact on DKK1 and total adiponectin concentrations compared to *ob/ob* mice, suggesting that these factors are not responsible for the myeloma-permissive environment generated by HFD. However, diet-induced obesity was found to significantly increase serum IGF-1, while no significant difference in IGF-1 concentration was observed in *ob/ob* mice. This suggests that the increase in IGF-1 is specific to diet-induced obesity and may be contributing to the development of myeloma. IL-6 was not found to be altered in either *ob/ob* mice or HFD mice, but was significantly increased in tumor bearing mice on HFD, suggesting that it came from myeloma cells, or that MM cells stimulated its increase in neighboring cells (58).

Increased myeloma incidence and development under obese conditions was also observed using a second diet-induced obesity (DIO) C57BL/6J mouse model (Figure 1B) (59). In this study, mice were injected with murine myeloma Vk12598 cells (which express high levels of *Myc*) into the femurs (intrafemoral). Tumor burden assessed with the serum levels of M-proteins was significantly higher in DIO mice compared with control diet mice. DIO mice also exhibited increased numbers of marrow and spleen-infiltrating CD138+ myeloma cells, as well as enlarged spleens (59). In a third model, within this same publication, Yang et al. used luciferase-labeled ARP-1 human myeloma cells (Figure 1C), mixed with purified mature adipocytes obtained from normal BM-derived mesenchymal stromal cells (MSCs) (59). Those were then injected subcutaneously into NOD-scid $IL2RG^{null}$ (NSG) mice fed with irradiated rodent diet. They found that adding human or mouse adipocytes to myeloma cells increased the levels of bioluminescent activity and tumor weights. Similar results were observed with intrafemoral injections of MM.1S or ARP-1 in SCID mice (Figure 1D). In addition, whether the murine adipocytes came from normal diet mice or HFD mice differentially affected tumor growth; higher bioluminescent activity was observed in the flanks of the mice injected with adipocytes isolated from HFD vs control diet mice (59).

4.2 Bone marrow adipose tissue

Obesity is known to cause an increase in BMAT in mice and humans (Figure 2), highlighting the potential for increased or altered BMAT to be a major link between obesity and MM (62–64). One of

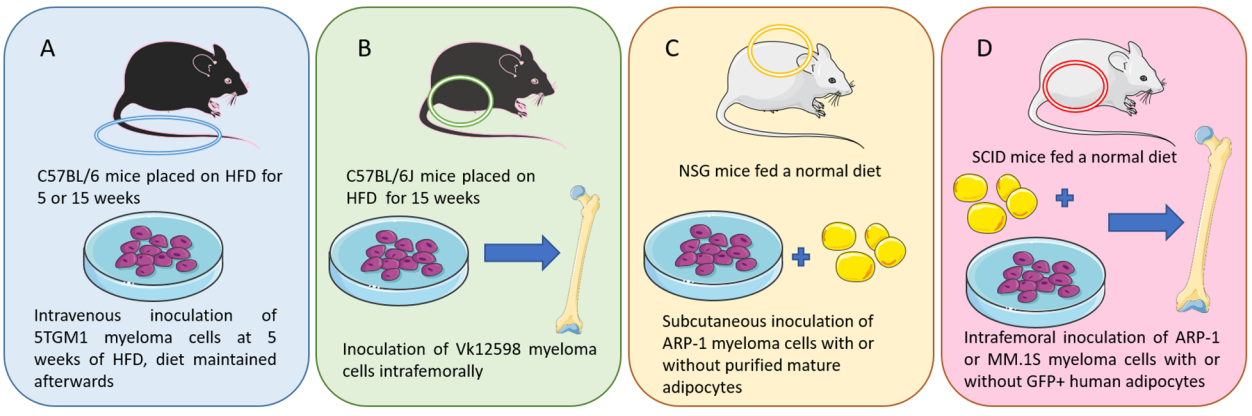

FIGURE 1
Murine models of multiple myeloma: **(A)** I.V. injection of 5TGM1 MM cells in C57BL/6 mice on a high fat diet (a diet-induced obesity model) (58). **(B)** Intrafemoral injection of Vk12598 MM cells in C57BL/6J mice on a high fat diet (a diet-induced obesity model) (59). **(C)** Subcutaneous inoculation of ARP-1 MM cells and adipocytes in NSG mice (59). **(D)** SCID mouse femurs injected with MM cells (ARP-1 or MM.1S) or co-injected with MM cells and GFP-labeled mature human adipocytes (60). Figure includes modified templates from Servier Medical Art.

FIGURE 2
Role of obesity in Multiple Myeloma: MM, Multiple Myeloma; ACSS2, Acetyl-CoA Synthetase 2; IRF4, Interferon Regulatory Factor 4; IL-6, Interleukin-6; AKT/STAT, Protein Kinase B/Signal Transducer and Activator of Transcription; TNF-α, Tumor Necrosis Factor-α; NF-kβ, Nuclear Transcription Factor kβ; NGF, Nerve Growth Factor; mTOR, Mechanistic/Mammalian Target of Rapamycin; 4EBP1, Eukaryotic Translation Initiation Factor 4E-Binding Protein; CRP, C-Reactive Protein.

the first studies to investigate a potential link between BM adipocytes and MM cells was from Caers et al., where 5T33MM murine myeloma cells and the human MM5.1 cell line were used (65). In this work, normal and concentrated conditioned medium (CM) from BM fibroblasts, BM adipocytes and peripheral adipocytes were added to 5T33MM cells, and their DNA synthesis was measured. A significant increase in DNA synthesis in MM cells was detected in response to BM adipocyte media compared to the control. Direct cell-cell contact between MM cells and adipocytic cells was also found to increase DNA synthesis significantly in murine 5T33MM cells. Fluorescence activated cell sorting (FACS) analysis of caspase-3 activity also showed that adipocytic cells protect MM cells from apoptosis. The migration assay revealed an enhanced migration of murine MM cells towards concentrated CM of BM adipocytes. Therefore, BM adipocytes affect proliferation, apoptosis and migration of MM cells (65). However, as MM cells invade the BM, BM adipocytes tend to disappear and change (eg. display a SASP, senescence-associated secretory phenotype, or become reprogrammed in other ways to induce bone resorption) during the disease development (66–71). This suggests that a bi-directional relationship exists between adipocytes and MM cells, and that the main role of adipocytes may be in the initial stages of the disease.

A combination of both *in vitro* and *in vivo* methods have been used to test the hypothesis that an increase in adipocyte quantity promotes the progression to MM. In 2016 Trotter et al. (72) were among the first to show that the BM from patients having MM contained more preadipocytes and larger mature adipocytes than normal BM. They was also found that preadipocytes and mature adipocytes secrete many molecules that support the growth of MM cells in the BM and recruit MM cells *via* both stromal cell-derived factor-1-a and monocyte chemotactic protein-1. In addition, CM from mature adipocytes augmented MM growth, and co-culture with preadipocytes resulted in increased MM cell chemotaxis *in vitro*. This supported the importance of adipocytes on MM progression and suggested they represent a specific target in the BM microenvironment (72). In 2019, using the C57BL/KaLwRij murine model of myeloma, BM adiposity was found to be increased in early stage myeloma, while bone marrow adipocytes (BMAds) were localized primarily along the tumor-bone interface at later stages of disease (69). Myeloma cells were found to uptake BMAd-derived lipids *in vitro* and *in vivo*, although lipid uptake was not associated with the ability of BMAds to promote myeloma cell growth and survival (69). The Yang laboratory has shown that adipocytes activate autophagy in myeloma cells, *via* the STAT3 signaling pathway, leading to chemoresistance and reduced apoptosis using eloquent *in vivo* and *in vitro* studies (60). Our work has also found that BMAT induces drug resistance in MM cells (70). New, tissue engineered 3D models of BMAT have also shown that BM adipocytes shrink with MM cell co-culture; these models may be useful to

recapitulate tumor-host interactions in more physiologically relevant conditions than can be achieved with 2D cultures, but with more control than possible *in vivo* (73, 74).

MM cells use mitochondrial-based metabolism as well as glycolysis in the BM (75). It was recently reported that intercellular mitochondrial transfer from neighboring nonmalignant BM stromal cells to MM cells *via* tumor-derived tunneling nanotubes (TNT) can change cellular reliance on oxidative phosphorylation, and hence BMAds could not only provide fatty acids, adipokines, and other fuel, but also, potentially mitochondria, which could affect MM cell survival and growth (75). This is an area open for interrogation.

4.3 Lipid mediators and metabolism-related enzymes

The roles of lipids and other metabolites in patient serum is also under investigation as a mechanism by which obesity may affect MM. For example, PI resistance may be partially mediated by oxidized LDL, a central mediator of atherosclerosis that is elevated in obesity (76). Oxidized LDL suppressed the boronic-acid based PIs (bortezomib and ixazomib) mediated killing of human MM cell lines through both proteasome inhibition and pro-apoptotic signaling (76). This implies that patients with metabolic syndrome (obesity, insulin resistance, dyslipidemia, and hypertension) could see deepened clinical response to these PIs when supplemented with cholesterol-lowering therapy. However, this is not supported by a 2008 study that found total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in patients with MM were significantly lower than the healthy controls (77). Similarly, a new 6-prognostic factor model was constructed based on Lasso regression to assess the following serum lipids in MM patients: triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), lactate dehydrogenase (LDH), Apolipoprotein B (Apo B) and Apo B/Apolipoprotein A1 (Apo A1) ratio (78). Together, these created a prognostic model, identified through univariate and multivariate Cox analysis, which exhibited better accuracy than International Staging System (ISS) and Durie and Salmon (DS) stage for 5- and 10-year OS. Data from the model support ApoB and the ApoB/ApoA1 ratio as being associated with shorter OS, but total cholesterol and HDL-C levels with being associated with longer OS (78). Still, the direction of the causal relationships between tumor development and changes in serum lipid levels is not known from the current data, and compensation mechanisms may be at work. Interestingly, *in vitro*, accumulation of lipids in MM cells was induced by PI treatment, and lipid-lowering drugs combined with a PI exerted a synergistic killing effect on myeloma cells (79).

Differences in circulating lipids or other metabolites may also affect tumor cell metabolism, and lipid metabolism within MM cells, in normal or high BMI patients, is of great interest. In a study analyzing myeloma cells isolated from obese patients (59), the involvement of the acetyl coenzyme A (Acetyl-CoA) metabolic process in obesity-associated myeloma growth was observed. The production of Acetyl-CoA from acetate is primarily dependent on Acetyl-CoA synthetase 2 (ACSS2), the enzyme which catalyzes the synthesis of Acetyl-CoA from short chain fatty acids. It was found that *ACSS2* was highly expressed in malignant plasma cells from MM patients. This expression was even higher in obese patients, and a correlation between *ACSS2* expression and patient BMI was established. In subsequent experiments, adipocyte-secreted angiotensin II was identified as the direct cause of this increased expression of *ACSS2*. ACSS2 interacts with the oncoprotein interferon regulatory factor 4 (IRF4), and enhances IRF4 stability and IRF4-mediated gene transcription through activation of acetylation. IRF4 plays a main role in B-lymphocyte development and acts as a transcription factor, regulating the expression of genes supporting myeloma growth and survival. As the use of an ACSS2 inhibitor reduced myeloma growth both *in vitro* and in a diet-induced obese mouse model, the critical role of ACSS2 in MM was verified (59). Another fatty acid metabolism gene, fatty acid elongase 6 (ELOVL6), was recently shown to regulate PI resistance in MM (80). We have found that the ACSL and FABP (fatty acid binding proteins) families of are other, novel potential targets in MM (81–83).

Bioactive lipid mediators may also be new targets in MM. Recent work from the Lynch lab has shown that knockdown of the acid ceramidase enzyme, ASAH1, which typically breaks down lipids of the ceramide class, led to reduced conversion of ceramide to sphingosine 1-phosphate (S1P) and decreased expression or activity of the anti-apoptotic proteins (MCL-1, BCL2 and BCL-xL) along with increases in pro-apoptotic proteins (BIM and NOXA) (84). Notably, ASAH1 knockdown also significantly sensitized the MM cells to PI treatment (84). In further support of this, S1P activation has been shown to contribute to proliferation and survival of MM cells (85). More information on targeting lipid metabolism in MM cells can be found here (86), and a review of metabolic changes in the BM microenvironment to relate to MM progression can be found here (87).

4.4 Adipokines

In addition to angiotensin II, as mentioned above, adipose tissue expresses and secretes multiple bioactive peptides called adipokines that act both on the local and systemic level. Adipokines regulate a broad range of activities including angiogenesis, oxidation, and cellular signaling. During metabolic diseases, including obesity, increases in adipose tissue result in subsequent increased production and secretion of many adipokines, with adiponectin a notable exception (88, 89). Leptin, an important regulator of caloric intake and metabolic function, is elevated in obese humans (89). It has been shown to be highly correlated with BMI in mice, and weight loss due to food restriction causes a decrease in the leptin levels (90). Caers and colleagues confirmed that adipocytes were the only cells within the MM microenvironment to secrete leptin, by RT-PCR and ELISA (65). Leptin also appears have proliferative and anti-apoptotic effects in myeloma cells (61, 91). Leptin has been shown to be responsible for reducing the anti-tumor effects of chemotherapy *via* activation of AKT and STAT3 pathways, as well as upregulating Bcl-2 expression and inhibiting caspase-3 activation in MM cells *in vitro* (61).

Adiponectin is one of most highly expressed substances in adipocytes, and was found to be decreased in obese patients (92). In a case-control study, the role of serum adiponectin in MM was investigated by analyzing blood samples collected from 73 patients

with histologically confirmed MM and 73 non-tumor bearing controls. Lower serum adiponectin levels were associated with higher risk of MM by bivariate analysis (93). While investigating whether circulating levels of total adiponectin and high molecular weight (HMW) adiponectin were associated with MM risk among 174 MM patients, an inverse relationship between total and HMW adiponectin levels and subsequent risk of MM was shown (94).

Much work from the Edwards lab has supported the anti-myeloma role of adiponectin in MM (95). Recently, from the Edwards lab, myeloma cells were found to downregulate adiponectin specifically in BMAds but not in white adipocytes (69). They found the ability of myeloma cells to downregulate adiponectin was dependent in part on TNF-α which was significantly correlated with tumor burden in the BM plasma of myeloma-bearing mice. To investigate how TNF-α downregulates BMAd-derived adiponectin, transwell co-culture of myeloma cells with BMAds was used, and it revealed an increase in activation of JNK, p38MAPK and ERK1/2, pathways that are implicated in TNF-α mediated suppression of adiponectin in WAT (69). Mouse recombinant TNF-α induced a significant decrease in *Adipoq* expression and in secreted adiponectin in ST2-derived mouse BMAds. Conversely, the addition of a neutralizing antibody to TNF-α in myeloma/BMAd co-cultures blocked the suppression of adiponectin and prevented the reduction in BMAd number. This work demonstrated that myeloma cells down-regulate adiponectin in BMAds *via* TNF-α (69). Adiponectin signaling may also help reduce bone pain, through a TNF-α-NF-kβ-adiponectin axis that regulated nerve growth factor (NGF) and pain signaling in MM (96). A recent study also found a correlation between adiponectin and markers of MM bone disease and further investigated a potential mechanism of action of adiponectin on the differentiation and maturation of osteoclasts in MM (97). Flow cytometry was used to detect the expression of adiponectin receptor 1 (AdipoR1) and the phosphorylation of the mechanistic target of rapamycin kinase (mTOR) and eukaryotic translation initiation factor 4E-binding protein (4EBP1). It was found that adiponectin inhibits the differentiation and maturation of osteoclasts by increasing the expression of AdipoR1 and reducing the phosphorylation levels of mTOR and 4EBP1 in patients with MM (97).

Data on the role of resistin do not clearly define the role of this adipokine in MM. In 2017, Pang et al. showed that resistin contributes to muti-drug resistance in MM cells by inhibiting cell death and upregulating ATP-binding cassette transporter expression (98). However, lower serum resistin levels correlated with higher risk of MM in one clinical study, adjusted for BMI and other factors, but it is possible that resistin levels were decreased in a compensatory/response mechanism in this case (93). Thus, more research into the roles of resistin, and other adipokines, in MM would be valuable.

4.5 Hormones and inflammatory cytokines

Obesity is often characterized as a hyperinsulinemic state. Insulin and IGF-I are potent growth and survival factor for MM cells (96). MM cell lines express IGF-I, IGF-II, and insulin receptors; insulin and IGFs can protect MM cells from dexamethasone-induced apoptosis and thus play a role in maintenance of the malignant clone (99). Work from the Rudikoff laboratory studied the IGF-I signaling cascade in 8 MM cell line; in addition to inhibiting apoptosis, IGF-I was found to activate the MAPK pathway, resulting in proliferation. Moreover, *in vivo* administration of IGF-I in SCID mice inoculated with the OPM-2 cell line led to a tumor growth rate twice as high as in the controls (100, 101). Inhibition of the IGF-I pathway was not found to change the proliferative effect of IL-6, and IL-6 and IGF-I were found to activate different downstream signaling molecules. Thus, IGF-I was found to act as a survival and proliferation factor for MM cells by stimulating an IL-6 independent signaling cascade (102).

Free serum sex hormones, eg. estrogen and testosterone, are is increased in obesity in part due to a decrease in the sex hormone-binding globulin (SHBG) (103, 104). These hormones interact with numerous pathways throughout the body that likely affect MM. For example, estrogens have been shown to promote MM by enhancing the immunosuppressive activity of myeloid-derived suppressor cells (MDSCs) (105). The estrogen-responsive gene microtubule-associated serine/threonine kinase family member 4 (MAST4) was also recently found to be a critical factor in MM-induced bone disease (106). Still, the relationship between estrogens and MM is not completely clear, because another study found that activation of estrogen receptors, which are highly expressed by MM cells, blocked interleukin-6-inducible cell growth of human MM cells (107).

The state of chronic inflammation associated with obesity is related to high levels of inflammatory cytokines such as IL-6, C-reactive protein (CRP), and TNF-α. IL-6 has been long known to be involved in inhibition of MM cell apoptosis and promotion of their survival (108). Interactions of IL-6 with adhesion molecules, cytokines, such as transforming growth factor beta-1, tumor suppressor genes and oncogenes, lead to the survival of malignant plasma cells (109). In addition, IL-6 is also suggested to cause drug resistance using epigenetic modulation proteins. IL-6 also enhances DNA methyltransferase-1, and thus promotes methylation and deactivation of p53, enabling MM cells to avoid apoptosis (110). CRP is a polypeptide protein secreted by hepatocytes in response to IL-6 and represents an indicator of IL-6 production. TNF-α induces expression of adhesion molecules on MM cell lines and on BM stromal cells leading to increased binding of MM cells to BM stromal cells and increased IL-6 secretion (99). The direct and indirect roles of other immunomodulatory interleukins, such as IL-10 (111), and the IL-12 family cytokines (112, 113), the gut microbiome (known to change based on diets and obesity) and adipocyte-derived chemoattractants such as MCP1 (also known to change in obesity (112),) are multifactorial and would benefit from further investigation (114).

4.6 Nutrient uptake

Metabolic transformations are one main aspect of cancer, and targeting these transformations is one critical lead for cancer therapy. Cancer metabolism and phenotype rely on cell-intrinsic factors, such as metabolite availability in the tumor microenvironment (TME). A large range of cell types contribute to the metabolite composition of the TME, and are involved in tumor cell metabolism, interactions between cancer and non-cancerous cells, and whole body metabolic homeostasis (115).

In work from the Raje laboratory using murine MM cells and BM adipocyte coculture assays, MM-induced lipolysis in adipocytes *via*

activation of the lipolysis pathway was investigated (71). In this work, MM cells were shown to induce lipolysis in adipocytes using a glycerol secretion assay. The observation of an upregulation of the genes involved in fatty acid (FA) lipolysis, FA synthesis, and FA desaturation lead to the conclusion that an altered FA metabolism is induced in MM cells (71). In this study, murine 5TGM1 and human-derived OPM2 MM cells were exposed to fluorescently labeled FA and analyzed. Results indicated that the cellular machinery for FA transport are present in MM cells, and lipolysis-induced free fatty acids (FFAs) are transferred intracellularly into the MM cells, potentially altering FA metabolism. Upregulation of fatty acid transporters 1 and 4 on MM cells mediated the uptake of secreted FFAs by adjacent MM cells. The effect of FFAs on MM cells was studied by peritumoral delivery of arachidonic acid on a plasmacytoma MM.1S model in SCID mice. This experiment revealed an increased proliferation at lower concentrations and the induction of lipotoxicity, *via* ferroptosis, at higher concentrations of FFAs. The authors rightly concluded that the prevention of FFA uptake by MM cells could represent a potential target for myeloma therapeutics (71). A deeper understanding of the metabolic flexibility and requirements of tumor cells, and downstream tumor cell changes resulting from different metabolic pathways, would enable novel therapy development in MM and many other cancers.

4.7 The immune system

Currently, no studies have specifically examined a three-way link between obesity, the immune system, and myeloma progression, despite the fact that all of the above biological effects or consequences of obesity also have effects on immune cells. However, different pairs of this triad have been studied in depth in MM as we have described above (obesity and MM) and as others have [immune cells and MM (116)]. Fortunately, there are potentially relevant findings in other malignancies that may translate to MM. Here we discuss some of the most recent studies investigating effects of obesity on T cells and macrophages in the TME, which represents a promising direction of research. To better understand the impact of obesity on MM and design novel interventions, the roles of other immune cells should also be investigated, especially now that immunotherapies are becoming so prevalent in MM treatment clinically.

4.7.1 HFD induced obesity impairs CD8+ T cell function in the murine TME

One main feature of tumor cell metabolism is increased nutrient consumption to meet energetic, anabolic and pro-survival demands. As activated T cells are highly proliferative and rely on metabolic pathways to function, tumor cell metabolism and anti-tumor immunity are tightly related. In 2020, Ringel et al. investigated how obesity shifts the metabolic landscape of the tumor microenvironment to inhibit T cell function and promote tumor growth (117), and though they did not use myeloma cells in this work, the possibility of their findings to apply to MM as well remains.

In the Ringel et al. work, to model human obesity in mice, C57BL/6J mice were put on either CD or HFD at 5 weeks of age, and for 8-10 weeks. They were injected with syngeneic MC38 colorectal adenocarcinoma cells, which grew more quickly in the HFD mice than in the CD mice. Flow cytometry was used to profile tumor-infiltrating immune cell populations 10-14 days after inoculation. HFD was found to reduce the number and functionality of intratumoral CD8+ T cells (117). Single-cell profiling revealed that immune cells in the TME go through metabolic changes in response to HFD, and the differences are distinctive in the T cells. Although CD8+ T cells are found within HFD tumors, it seems that HFD changes metabolic niche interactions within tumors and impacts local T cell infiltration patterns. These metabolic adaptations differ from the ones affecting tumor cells, which lead to altered fatty acid partitioning in HFD tumors, impairing CD8+ T cell infiltration and function. HFD MC38 tumor cells rewire metabolism to increase fatty acid uptake and oxidation. Indeed, HFD decreased expression of prolyl hydroxylase 3 (*PHD3*), a critical metabolic regulator, in MC38 cells. Restoring *PHD3* expression in tumor cells was found to be sufficient to alter nutrient availability in the TME. Investigating the effect of *PHD3* overexpression on tumor growth *in vivo*, data showed that maintaining high PHD3 expression in MC38 tumor cells improved the anti-tumor T cell response in HFD mice. Analysis of other human cancers revealed similar changes in CD8+ T cell markers, suggesting interventions that exploit metabolism may improve cancer immunotherapy (117).

The effects of obesity on CD8+ T cells were also investigated by Dyck et al. in mouse models and patients with endometrial cancer (118). It was also found that obesity enhances tumor growth and reduces CD8 T cell infiltration, proliferation, and function in the tumor. Suppression of CD8+ T cell infiltration in obesity was associated with a decrease in chemokine production such as IFN-γ. Tumor-resident CD8+ T cells were also found to be functionally suppressed in obese mice, due to a suppression of amino acid metabolism. In fact, CD8+ T cell activation requires the activity of the amino acid transporters SLC7A5, and their activity in MC38 tumors in obese mice was found to be significantly reduced. Glutamine levels were also decreased in mice on HFD, and as it is essential for CD8+ T cell function and is a substrate for SLC7A5, low levels of glutamine could impair CD8+ T cell function in obesity. The immune checkpoint PD-1, which is frequently up-regulated on dysfunctional T cells in tumors, was also found to be more highly expressed in MC38 tumors. Immunotherapy using anti-PD-1 partially restored CD8 metabolism and anti-tumor immunity (118).

4.7.2 Macrophages and tumor cells

Tumor-associated macrophages (TAMs) possess distinct phenotypes. While M1 macrophages have a pro-inflammatory (anti-tumoral) function, M2 macrophages have an anti-inflammatory (pro-tumoral) function. Studies have shown that the polarization of TAMs towards an M1 or M2 phenotype is driven by environmental factors such as cytokines, chemokines and other soluble factors secreted by the neighboring cells. Polarization towards the M2 state has been found to be correlated with a lack in p53 (115). The discoveries regarding the metabolic profile of macrophages represent an interesting therapeutic target: promoting a switch to an M1, anti-tumoral phenotype.

Like T cells, TAMs also compete with their neighboring cells for glucose. Glycolytic activity in TAMs has mainly been associated with tumor regression (115). Moreover, hypoxic TAMs have increased expression of the mTOR negative regulator REDD1 and consequently

display decreased glycolysis. Lactate produced by tumor cells also has a critical function in signaling and TAM polarization as it induces a pro-tumoral M2 phenotype (115). Glutamine metabolism in TAMs has also been linked to a pro-tumoral phenotype (115). Monocytes have also recently been shown to respond to HFD in mice, in work from the Klip laboratory: this group found that short-term HFD changed BM cellularity, resulting in local adipocyte whitening, driving a gradual increase and activation of invasive Ly6C^{high} monocytes (119). These monocyte changes preceded a rise in adipose tissue macrophages during HFD in the mice. Moreover, skewing of the BM towards a preponderance of Ly6C^{high} monocytes was preceded by monocyte metabolic reprogramming towards glycolysis, reduced oxidative potential and increased mitochondrial fission. Thus, obesity, or general metabolic state, likely also affects tumor progression through influences on monocyte or macrophage metabolism.

In a recent study by Micallef et al. the C1q/TNF-related protein family member C1qtnf3 was identified as one of the most upregulated genes responsible for secreted proteins in tumor-associated adipose tissue, especially in diet-induced obese mice (120). Macrophage accumulation in tumor-associated inguinal adipose tissue was found to be inhibited by the administration of C1QTNF3 neutralizing antibodies, but tumor growth was unaffected. Moreover, C1QTNF3 treatment of M2 macrophages stimulated the ERK and Akt pathway, increasing the polarization towards the M1 state. These results suggest that macrophages could be recruited to adipose tissue with increased C1QTNF3 production (120). In sum, aiming to redirect a polarization of TAMs towards an M1 phenotype by affecting metabolism, or other signaling pathways, represents a promising therapeutic target.

5 Discussion

As evidenced by the scant publications available in the myeloma field, modeling the contributions of obesity to MM progression in mice has been difficult (57). These challenges are compounded by the fact that many myeloma studies utilize human MM cells, which require the use of immunocompromised (eg. NOD/SCID, SCID-beige, or nude) mice, which do not exhibit robust responses to DIO regimens, and are lacking a full immune cell repertoire. Indeed, a thorough investigation of changes in tumor cells and BM immune cell milieu induced by obesity that support MM disease progression has not been executed. Most studies of obesity on MM rely on direct injection of mouse tumor cells into the bones (59) of mice on a HFD, due to the unpredictability of mouse tumor cell engraftment when injected systemically, and often result in growth in locations other than the bone.

The Vk*MYC mouse myeloma cell line Vk12598, which grows in C57BL/6 mice, does induce osteolysis when injected systemically into mice (121), which is even more pronounced when the cells are injected intrafemorally (34). So far, as we described above, this model has only shown a response to DIO when the Vk12598 cells are injected intrafemorally, which is useful but does not recapitulate the series of steps MM cells take in disease progression (ie. extravsation, survival through the circulation, intravasation, homing to the bone marrow) (59). However, murine eGFP+/5TGM1 cells, originally derived from the C57BL/KaLwRij mice (122), can spread to the bone from circulation and cause extensive osteolysis, as we have shown (123). However, they can also spread to other locations, such as the spleen, which poorly models MM clinically (59). Still, these cells have proven useful in a MM DIO model using IV injection (58, 59), and hopefully, using this or a similar immunocompentent, DIO model, the mechanisms of obesity's effects on MM can be better elucidated. We now know obesity alters the composition of the BM (124), as well as the genes expression profiles of the cells within the niche (125). Obesity is correlated with an increase in BMAds in mice (13) and humans (126), which are known contributors to systemic adipokines such as leptin (pro-myeloma) (94) and adiponectin (anti-myeloma) (95), however the relationship between MM and BMAds has only begun to be investigated. Recent studies by our lab and others have demonstrated significant effects of MM cells on BMAds including decreased lipid content (66, 68–70), increased expression of inflammatory cytokines (66, 70), and decreased adiponectin (69, 70), with myriad implications for direct interactions with MM cells, as well as the vicious cycle of MM-induced bone disease. Whether these myeloma-associated adipocytes are involved in homing, engraftment, or *in vivo* drug resistance should be further investigated in animal models going forward, since we and others have shown that adipocytes promote drug resistance in myeloma cells *in vitro* (60, 70, 98).

Obesity, like aging (127), is now being linked to senescence in the BM microenvironment (125) and cellular senescence and cancer susceptibility are correlated in a number of conditions (128), begging the question of whether aging and obesity-induced senescence in the marrow might actually drive myelomagenesis. Recent data from the Weivoda laboratory support this idea, based on clinical data and mouse models showing that MGUS and smoldering MM plasma cells are in a senescent-like state, and data that targeting senescence in these early MM diseases can reduce plasma cell numbers in the MGUS model (129). Moreover, *in vitro* studies suggest that myeloma cells might induce senescence in the marrow in mesenchymal stromal cells (14, 20, 21) and BMAds (13), however the relationship between senescent cells and myeloma is still not completely understood. Promising preclinical studies in both aged (127) and obese (130) mice, and preliminary reports from a clinical trial (131), demonstrate efficacy of senolytic therapies to target and clear senescent cells in obesity models and aging. Future studies should investigate a role for senolytics in combination with traditional myeloma chemotherapies and treatments, particularly since MM therapies often induce senescence.

6 Conclusion

Obesity has been implicated in myeloma risk and transformation from MGUS to MM, as well as MM mortality. Direct mechanisms tying obesity-driven increases in adipose tissue to tumor cell proliferation and survival through secretion of cytokines such as leptin and IL-6, or to myeloma cell drug resistance are being unveiled. The three-way relationship between myeloma cells, adipocytes, and the immune system, and the potential for metabolic- or senescence-focused therapies should be examined going forward. Preclinical studies, including the establishment of a reliable, bone-homing, immunocompetent DIO model are critical to understanding the

complex molecular mechanisms at play in the obesity-myeloma relationship.

Author contributions

CM-M and RI researched and wrote the article. HF and MR researched, wrote, reviewed and edited the article. All identified the theme of the review. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Obesity and cancer: Mouse models used in studies

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There is increasing evidence that obesity is associated with the occurrence and development of malignant tumors. When studying the relationship between obesity and malignant tumors, it is very important to choose an appropriate animal model. However, BALB/c nude mice and other animals commonly used to study tumor xenograft (human-derived tumor cell lines) transplantation models are difficult to induce obesity, while C57BL/6 mice and other model animals commonly used for obesity research are not suitable for tumor xenograft transplantation. Therefore, it is difficult to replicate both obesity and malignancy in animal models at the same time. This review summarizes several experimental animal models and protocols that can simultaneously induce obesity and tumor xenografts.

KEYWORDS

obesity, cancer, diet-induced obesity, mouse model, preclinical disease model

Introduction

Over the past few decades, obesity has become a growing global health problem. From 1975 to 2016, the global prevalence of obesity nearly tripled, affecting 13% of the world's adult population (1). A large body of epidemiologic evidence shows that obesity is associated with the incidence and progress of several cancers. According to the World Cancer Research Fund's Third Expert Report, obesity is an important risk factor for many types of cancer (2). The mechanisms linking obesity and cancer development remain unclear. The impact of obesity on human health may take decades to become apparent. Therefore, the use of experimental animals to study the effects of obesity on cancer is of great importance for the discovery of the phenomenon and the study of the mechanism.

Researchers often use preclinical animal models to study the relationship between obesity and disease. Because gene knockout and transgenic technology cannot fully reflect the pathogenesis and pathogenic factors of obesity, the current modeling method is still based on food inducing. Immunodeficient mice are widely used in cancer research. Because xenografts can be performed, they provide researchers with insight into the growth, invasion, and metastasis of human tumor cells. In addition, researchers have also created

several types of genetically engineered mouse models (GEMMs) that can spontaneously develop cancer.

However, replicating both obesity and malignancy in laboratory animals is extremely difficult. Animals commonly used in obesity models cannot engraft heterolytic tumors. On the other hand, it is difficult to induce obesity in animals commonly used in cancer models. This situation leaves researchers with limited options. In this review, we discuss the mouse model and related experimental strategies for obesity and cancer research.

Obesity model

Animal models of obesity are diverse and include both mammalian and nonmammalian species. Non-mammals have certain limitations due to major anatomical and physiological differences from humans (3). Therefore, mammals are usually considered the ideal animal model for obesity research. Among mammals, mice are most used. This is because of their small body size, high reproductive capacity, relatively short life cycle, and relatively easy genome editing (4, 5).

Diet-induced obesity

Diet-induced obesity (DIO) is an important model of obesity and results from excessive consumption of a high-fat diet (HFD), which usually contains 45-60% fat (6). DIO can simulate the development of human obesity better than genetic models (7, 8) and commonly use the mouse as the model (9). Consumption of HFD can lead to central obesity and insulin resistance in mice and is a good research alternative to mimic diet-induced obesity in humans.

Mouse species

Among inbred mice, C57BL/6J, BALB/c, KM, and ICR mice are commonly used to reproduce DIO models (10). Other inbred strains, such as SWR/J and A/J mice, are less sensitive to high-fat diets and related complications (11). The C57BL/6J has the advantage of short modeling time and stable metrics, so it is the most widely used. The C57BL/6J is more susceptible to fat accumulation, weight gain, and glucose metabolism disorders when fed a high-fat diet, as manifested by significant changes in abdominal fat weight, Lee's index, and adipocyte volume.

Age and sex of the mice

The weight of C57BL/6J mice gradually increased with age, reaching the peak at approximately 9 months (12). Compared with the younger mice, the older ones (22 months old or older) had less muscle and more fat (13). Male mice are often used in experimental studies to induce obesity because they are more sensitive to high-fat diets and are prone to diet-induced insulin resistance and abnormal glucose tolerance (14, 15). Compared with male mice, female mice gain weight slowly, have a low obesity rate, and are generally resistant to high-fat diet-induced obesity (16, 17). However, because brown adipose tissue is easier to observe in female mice, female C57BL/6J mice are generally used to study the role of brown adipose tissue in energy metabolism (18).

Monogenic obesity model

Two types of spontaneously obese mice based on C57BL/6J were identified at the Jackson Laboratory, ob/ob mice in 1950 and db/db mice in 1965. The ob/ob mice lack functional leptin, whereas the db/db mice lack functional leptin receptors. Both types of mice exhibit overeating and are the primary mouse models for studies of monogenic obesity (4, 7). The ob/ob mice have a single base pair mutation in the ob gene, resulting in the absence of functional leptin, increased body weight, hyperphagia, and a low resting metabolic rate. On the other hand, due to a defect in the leptin receptor, leptin signaling is impaired in the db/db mice, resulting in significantly higher serum leptin levels. Therefore, the treatment of reorganization is sufficient to make ob/ob mice normal (19), but it is not effective for db/db mice (20). In addition, the two types of mice are the same in obesity, hypogonadism, and growth hormone (GH) deficiency (4, 6).

Monogenic obesity models have become important research tools in modern drug discovery. The ob/ob mouse is commonly used to evaluate the efficacy of new obesity drugs in overcoming the obesity phenotype caused by overeating (21), and db/db mice are commonly used to study the efficacy of antidiabetic drugs (22). These models require only short-term rather than long-term feeding to induce obesity. However, monogenic models generally do not represent the full pathogenesis of human obesity. Monogenic obesity in humans accounts for only a small proportion of obesity, and a few of human obesity can be explained by mutations in leptin or leptin receptors alone.

Polygenic obesity model

Compared to the monogenic model, the polygenic model can better simulate the pathogenesis of human obesity. The C57BL/6J mouse is the most used obese mouse model, which is susceptible to obesity induced by overeating. However, only 60% of C57BL/6J mice gain weight under high-fat diet conditions. The susceptibility of C57BL/6J mice to diet-induced obesity is typically characterized by changes in plasma insulin and leptin levels and insulin sensitivity at 6 weeks of age (23). New Zealand obese (NZO) mice are polygenic inbred mice predisposed to obesity and type 2 diabetes. Unlike C57BL/6J mice, NZO mice can gain weight on a standard diet (24).

Tumor mouse model

Mice have similar biological, physiological and pathological characteristics to humans and exhibit a high degree of genetic similarity, making them an ideal animal model for the study of tumors. Much of the current understanding of human cancer

characteristics are based on long-term *in vitro* culture of tumor cell lines and their inoculation into mice.

Tumor implantation model

Currently, most tumor implantation models used in basic or translational oncology research are based on established cell lines (25). They usually function as allografts of primary mouse tumors or xenografts of human tumors. In both types of models, cancer cells can be injected orthotopically or ectopically (mainly subcutaneously) and subsequently monitored for growth or metastasis (intraperitoneally, intravenously, or intracardially).

Since 1950, allografts have been used primarily as a preclinical model for drug development and cancer therapy (26). For example, researchers established the leukemia model using male DBA/2 mice and found that AZD2014, an mTORC1/2 inhibitor, inhibited the growth and proliferation of L1210 leukemia cells (27). The toxicology of some cytotoxic drugs has also been successfully studied in allograft models. However, allograft tumor models are of limited value for the study of human tumors. Therefore, xenograft tumor models have replaced allograft tumor models as the primary tool for preclinical drug testing since 1990.

Tumor ectopic transplantation model

The discovery of the thymus-free nude mouse was a major breakthrough in cancer research, allowing human tumors to be replicated in xenogeneic experimental animals. Immunodeficient mice have remarkable xenograft success rates and are able to preserve the original tissue structure and function of human cancers. Representative immunodeficient mice include nude mice, severe combined immunodeficiency (SCID) mice, non-obese diabetic/SCID (NOD/SCID) mice, and NOD-SCID-IL2Rg$^{-/-}$ (NSG) mice (Table 1). SCID mice have been shown to be more

TABLE 1 Characteristics and application of common immunodeficient mice.

Mouse strains	Background	Characteristic	Application	Notes
Nude	BALB/c	Mutations in the Foxn1 gene result in thymic aplasia, lack of T cells, and no immunological rejection.	It plays an important role in tumor, immunity, drug safety evaluation and preclinical screening of drugs.	It is not suitable as a host for leukemia or lymphoma because human hematopoietic stem cells are not transplantable into nude mice.
CBA/N	CBA/H	Btk gene mutations, defective B lymphocyte function, absent humoral response.	It can be used in the bone marrow transplantation model and is an ideal tool for studying the production, function and heterogeneity of B lymphocytes.	The incidence of spontaneous tumors is low and rarely used in oncology studies.
Beige	C57BL/6	Beige gene mutations, defective NK cell development and function and impaired humoral response.	It is widely used in immunology research.	It is more sensitive to various pathogenic factors and needs a good SPF environment.
SCID	CB-17	Mutations in the Prkdc^{scid} gene result in V(D)J recombination *in vivo* and defects in the generation of T and B-cell.	It is a good candidate for the xenograft tumor, especially blood-derived tumor cells and initially used as recipients of human hematopoietic stem cell and peripheral blood mononuclear cell transplantation.	SCID mice are more prone to die from infections. Among a small number of SCID mice, a certain degree of immune recovery may occur in young adulthood.
NOD/ SCID	NOD	Prkdcscid gene mutation in NOD background. In innate and adaptive immune deficiency, various tumor cells can be implanted, with less rejection and graft-versus-host disease.	NOD-SCID mice accept allogeneic and xenogeneic grafts, making them a suitable model for cell transfer experiments. A high degree of immunodeficiency but low immune infiltration.	Spontaneous thymic lymphoma occurs, resulting in a shorter lifespan, which makes it unsuitable for long-term transplantation.
NRG	NOD	NOD background carrying Rag1^{null} and IL2rg^{null} gene mutations. Deficient in B, T and NK T cells.	Human hematopoietic stem cells containing CD34+ and PDX can be efficiently transplanted to establish transplanted humanized mice models.	More resistant to irradiation and genotoxic agents than Prkdcscid mice.
NSG	NOD	NOD background carrying Prkdc^{scid} and IL2rg^{null} gene mutations. Deficient in B, T and NK T cells.	It is widely used in humanized mouse models of immunology, drugs, viruses and tumors.	An internationally recognized animal model with the highest degree of immunodeficiency and most suitable for xenotransplantation. Low incidence of lymphoma, low immune infiltration, sensitivity to radiation.
BRG	BALB/c	BALB/c background carrying Rag1^{null} and IL2rg^{null} gene mutations. Deficient in B, T and NK T cells.	It is a super immunodeficient mouse that is useful for research on humanization, infectious diseases, autoimmune diseases and in xenograft assays.	May be an ideal animal model to replace SCID mice in the future.

Nude, Nude mice; PDX, Patient-Derived Xenograft; NK cell, Natural Killer cell; NOD, Non-Obese Diabetes; SCID, Severe Combined Immunodeficiency; NRG, NOD-Rag1$^{-/-}$-IL2rg$^{-/-}$; NSG, NOD-SCID-IL2rg$^{-/-}$; BRG, BALB/c-Rag1$^{-/-}$-IL2rg$^{-/-}$.

suitable for human cancer cell xenografts than nude mice and have advantages for studying the biology of human tumors *in vivo* and their response to therapy (28).

Ectopic transplantation typically inoculates human cancer cell lines or pieces of tumor tissue under the skin in the axilla, back and hind legs of mice. After subcutaneous inoculation, the tumor tissue is surrounded by a thick fibrous capsule and rarely metastasizes to adjacent tissues. Tumor growth can be easily observed and the treatment efficacy can be evaluated.

Tumor orthotopic transplantation model

The microenvironment of *in situ* implanted tumors is different from that of ectopically implanted tumors, and therefore their growth rates are different. Because growing in an optimal microenvironment, *in situ* implanted tumors generally exhibit more active proliferation, metastasis, and invasion, which better mimics the growth of tumors in the human body (29). Fu XY et al. orthotopically implanted human colon cancer cells in the colon of nude mice. The transplanted tumors almost exactly replicated the characteristics of the corresponding human cancer, which included local tumor growth, abdominal metastasis with peritoneal seeding, liver metastasis, lymph node metastasis, and intestinal obstruction (30). Carmelo Nucera et al. established an orthotopic model of human thyroid cancer using the anaplastic thyroid carcinoma cell line 8305C and observed tumor growth and metastasis (31). However, because the volume and number of tumors in the visceral organs are not easily measured, there are cases where tumor ectopic transplantation is more appropriate.

Tumor intravenous transplantation model

The above ectopic and orthotopic transplantation models, also called spontaneous tumor metastasis model. The method of injecting cancer cells directly into the blood to study their spread and metastasis is called experimental tumor metastasis model. The experimental metastasis model is used to study the growth of malignant tumors in distant organs. Intravenous injection can shorten the time of tumor formation in target organs. Inoculation *via* the tail vein is one of the most used methods in the experimental metastasis model. For example, Nan Huo et al. established a lung metastasis model for thyroid cancer by injecting TPC-1 cells into BALB/c nude mice *via* the tail vein (32).

Genetically engineered mouse model

In the 1980s, the development of transgenic and gene-targeting technologies in mouse embryonic stem cells facilitated the generation of GEMM. The most common ways to generate GEMM are to activate oncogenes or inactivate tumor-suppressor genes *in vivo* through the use of transgenic and gene targeting methods, such as knock-outs and knock-ins. Gordon et al. established the first transgenic mice in 1980, harboring randomly integrated oncogenes under the control of a tissue-specific promoter (33). The initial set of genetic engineering tools was set against the background of the emergence of genome-editing technologies such as restriction endonucleases, DNA cloning and sequencing, and then developed lentiviral vector, electrotransfection and microinjection techniques. In 2016, the single-base gene editing technology developed from CRISPR-Cas9 avoided DNA double-strand breaks and further expanded the scope of base editing. The innovation of gene editing technologies has significantly reduced the time needed to establish a GEMM (34). GEMM has been used in the study of colorectal cancer (35), renal cell carcinoma (36) and breast cancer (37). In addition, it can be used in preclinical trials for hormonal and targeted therapies as well as immunotherapy. PD-1 KO and PD-L1 KO mice have been exploited to develop drugs for cancer treatment (38).

Obesity-associated cancer model

In vivo animal models are important research tools to study the underlying mechanisms of the association between obesity and cancers. Among genetic models of obesity, mice deficient in leptin signaling are the most used. When mice were fed standard chow, the genetic model showed early-onset obesity and comorbid diseases such as insulin resistance and hepatic steatosis. Their main disadvantage is the exclusion of the factors other than leptin that may affect cancer cells and tumor microenvironment. For example, obesity accelerates the progress of Kras-driven pancreatic ductal adenocarcinoma, but not lung cancer (39).

The DIO mouse model is believed to mimic human obesity well and to explain the potential biological link between obesity and cancer. The DIO model was established by feeding mice a diet high in sugar, fat or both. While several feeding regimens have been developed, the most commonly used diets contain 30% to 60% kcal from fat, which is fed to the mice for 10 to 12 weeks prior to tumor formation.

Most obesity-related complications are due to inflammation (40). Chronic inflammation in adipose tissue, especially white adipose tissue (WAT), stimulates cancer progression through mechanisms such as altered levels of adipokines and inflammatory mediators, and insulin resistance (41–43). Short-term HFD feeding is difficult to obtain an ideal model sufficient to study the relationship between obesity and cancer (44). Therefore, long-term obesity models need to be established to simulate the relationship between human obesity and tumors. The feeding time of the HFD-induced obesity mouse model ranged from 4 weeks to 56 weeks, and 10 weeks to 12 weeks were usually selected (Table 2). DIO mice gain weight, increase fasting blood glucose levels, and develop obesity-related phenotypes such as hyperinsulinemia, insulin resistance, hepatic steatosis,

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TABLE 2 Overview of obesity-associated cancer model.

Obesity model	Mouse strains	Diet	Duration	Cancer model	Obese tumor phenotype/proposed mechanism	Ref
DIO	Nude	HFD (35% kcal from fat)	4 weeks	T (TE-1, 2.0×106 cells, subcutaneous)	Obesity Potentiates Esophageal Squamous Cell Carcinoma Growth and Invasion by AMPK-YAP Pathway	(45)
		HFD (40% kcal from fat)	16 weeks	T (SKOV3i.p-RPF, 5×10^6 cells in 2ml PBS, orthotopic)	Obesity Contributes to Ovarian Cancer Metastatic Success Through Increased Lipogenesis, Enhanced Vascularity, and Decreased Infiltration of M1 Macrophages	(46)
		HFD	16 weeks	T (PC3.pGIPZ/PC3.shCtBP1, 4.8×10^6 cells, subcutaneous)	Prostate Tumor Growth Is Impaired by CtBP1 Depletion in High-Fat Diet–Fed Mice	(47)
	C57BL/6	HFD (42% kcal from fat)	4 months	–	High fat diet promotes prostatic basal-to-luminal differentiation and accelerates initiation of prostate epithelial hyperplasia originated from basal cells	(48)
		HFD (42% kcal from fat)	40 weeks	GEMM (Alb-Cre; Ptpn2fl/fl)	Obesity Drives STAT-1-Dependent NASH and STAT-3-Dependent HCC	(49)
		HFD (60% kcal from fat)	Until endpoint	GEMM (MUP-uPA)	Endoplasmic reticulum stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development	(50)
			8 weeks	T (AsPC-1, 1×10^5 cells, orthotopic) GEMM (KPC mice)	Critical role for arginase 2 in obesity-associated pancreatic cancer	(51)
			9-14 months	T (Apc-null Lgr5-GFP^{hi} ISCs/ Lgr5-GFP^{low} progenitors cells, orthotopic)	High fat diet enhances stemness and tumorigenicity of intestinal progenitors	(52)
	NSG	HFD (60% kcal from fat)	7days	T (SCC-25, FaDu, Detroit-562, JHU-029, orthotopic)	Targeting metastasis-initiating cells through the fatty acid receptor CD36	(53)
	-	HFD (60% kcal from fat)	From 8 weeks until endpoint	GEMM (ThrbPV/+Pten+/−)	Inhibition of STAT3 activity delays obesity-induced thyroid carcinogenesis in a mouse model	(54)
			From 6 weeks until endpoint	GEMM (ThrbPV/+Pten+/−)	Diet-induced obesity increases tumor growth and promotes anaplastic change in thyroid cancer in a mouse model	(55)
	BALB/c	HFD (60% kcal from fat)	20 weeks	T (CRL-2947-Luc, orthotopic)	Elevated Leptin during Diet-Induced Obesity Reduces the Efficacy of Tumor Immunotherapy.	(56)
MOM	ob/ob	–	–			
DIO	C57BL/6	HFD (60% kcal from fat)	10 weeks	T (Pan02, AK4.4, orthotopic)	Obesity-induced inflammation and desmoplasia promote pancreatic cancer progression and resistance to chemotherapy	(57)
MOM	ob/ob	–	–			
	ob/ob db/db	–	–	T (Pan02, 2.5×10^5, subcutaneous)	Obesity potentiates the growth and dissemination of pancreatic cancer	(58)
	ob/ob db/db	–	–	GEMM (KC crossed with ob/ob)	Endocrine-Exocrine Signaling Drives Obesity-Associated Pancreatic Ductal Adenocarcinoma.	(39)

Nude, nude mice; HFD, high fat diet; T, transplant model; GEMM, genetically engineered mouse model; ISCs, intestinal stem cells; MOM, monogenic obesity model (unless otherwise listed, duration of feeding indicates feeding pattern before transplantation or induction of cancer).

hypertension, and dyslipidemia (59). Whether reversal of the obesity phenotype affects tumor prognosis is a key question in this field. Dietary pattern switching experiments have shown that once DIO is established, a low-fat diet (LFD) for a prolonged period, such as 5 weeks, is sufficient to reverse obesity-induced chronic inflammation and tumor progression (44, 52).

Nude mice used to establish tumor xenograft models, such as BALB/c, are generally difficult to induce obesity. Stemmer K et al. found that Foxn1 nude mice (B6. Cg-Foxn1nu/J) on a C57BL/6 background fed a high-fat diet under thermoneutral (33°C) conditions significantly increased their body weight (60), making them an excellent model for studying obesity and tumors.

Discussion

Obesity is an important risk factor for cancer. Significant attention has been paid to the underlying mechanism between the two diseases. Appropriate animal models replicating both obesity and cancer are highly needed to study their association. A brief review shows that there is currently no single ideal model for this type of research (Table 2). The models listed are good for studying tumor progression and metastasis, but there are also some shortcomings. They cannot determine how diet and obesity contribute to cancer initiation and be used to study cancer survivorship.

The mouse models utilize high-fat diets to achieve obese condition but the typical western diet that is most closely associated with obesity and cancer is composed of a dietary pattern comprised of high protein and fat but most importantly very high in refined sugars (61, 62). This particular dietary pattern is not similar to mouse models and although it would be difficult to replicate in models the shortcomings should be noted (63). Humans who are exposed to high carbohydrate diets will not only lead to weight gain and obesity, but exacerbate glucose/insulin homeostasis which could be an important underlying mechanism associated with the progression of cancer independent of obesity or perhaps in synergy (64). Furthermore, a western dietary pattern has been associated with inflammation (65–67) and this is another important exposure that is missing in most animal models of cancer.

When selecting an appropriate mouse model, factors such as obese phenotype, environmental stimuli, mouse strain and sex should be considered more fully. With the development of different mouse models, the combined application of multiple models makes cancer research more convenient and accurate. Recently, the emergence of a revolutionary CRISPR/Cas9 system has greatly enhanced the efficiency of precise gene editing in various GEMMs. However, the potential risk of off-target effects is a notable concern. An ideal cancer + obesity mouse model should be technically simple, quick in operation, easily reproducible, affordable and short in modeling. Further improvement of obesity-prone mice that can be implanted with human tumor cells will help decipher the mechanism by which obesity affects tumor initiation and progression.

Author contributions

JJ and YZ contributed the central idea and analyzed most of the data. B-TZ and J-YX wrote the initial draft of the paper, WW contributed to refining the ideas and carrying out additional analyses. All authors reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Correlation between pancreatic cancer and metabolic syndrome: A systematic review and meta-analysis

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Objective: Pancreatic cancer is a globally frequent cause of death, which can be caused by many factors. This meta-analysis was performed to assess the correlation between pancreatic cancer and metabolic syndrome (MetS).

Methods: Publications were identified by searching PubMed, EMBASE, and the Cochrane Library for studies published until November 2022. Case-control and cohort studies published in English that provided information on the odds ratio (OR), relative risk (RR), or hazard ratio (HR) of metabolic syndrome and pancreatic cancer were included in the meta-analysis. Two researchers separately retrieved the core data from the included Random effects meta-analysis was conducted to summarize the findings. Results were presented as relative risk (RR) and 95% confidence interval (CI).

Results: MetS showed a strong association with an increased risk of developing pancreatic cancer (RR1.34, 95% CI1.23–1.46, P<0.001), and gender differences were also observed (men: RR 1.26, 95% CI 1.03–1.54, P=0.022; women: RR 1.64, 95% CI 1.41–1.90, P< 0.001). Moreover, an increased risk of developing pancreatic cancer was strongly linked to hypertension, poor high-density lipoprotein cholesterol, and hyperglycemia (hypertension: RR 1.10 CI 1.01–1.19, P=0.027; low high-density lipoprotein cholesterol: RR 1.24 CI 1.11–1.38, P<0.001; hyperglycemia: RR 1.55, CI 1.42–1.70, P< 0.001). However, pancreatic cancer was independent of obesity and hypertriglyceridemia (obesity: RR 1.13 CI 0.96–1.32, P=0.151, hypertriglyceridemia: RR 0.96, CI 0.87–1.07, P=0.486).

Conclusions: Although further prospective studies are required for confirmation, this meta-analysis indicated a strong relationship between MetS and pancreatic cancer. Regardless of gender, a greater risk of pancreatic cancer existed in people with MetS. Patients with MetS were more likely to develop pancreatic cancer, regardless of gender. Hypertension, hyperglycemia, and low HDL-c levels may largely account for this association. Further, the prevalence of pancreatic cancer was independent of obesity and hypertriglyceridemia.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42022368980.

KEYWORDS

metabolic syndrome, pancreatic cancer, metabolic component, meta-analysis, pancreas

Introduction

Pancreatic cancer (PC) is a common malignant tumor type with the 12th-highest incidence rate among all malignant tumors (1). PC has a dismal prognosis, with a general five-year relative survival rate of 10%, and it is the fourth and sixth most widely occurring common cause of cancer-related mortality in China and the United States, respectively (2, 3).The risk factors are unclear, and PC may develop in patients with a family history of cancer as well as those who smoke, drink alcohol, are obese, or have diabetes (4).

The metabolic syndrome (MetS) has attracted considerable attention with regard to its association with cardiovascular risk factors, first proposed in 1988 (5).Dyslipidemia, central obesity, poor glucose tolerance, insulin resistance, type 2 diabetes, and hyperinsulinemia are some abnormal metabolic parameters characterizing MetS (6). These parameters are typically assessed using the following indicators: blood pressure, fasting plasma glucose level, waist size, high-density lipoprotein cholesterol (HDL-c) levels, and triglyceride level (7). MetS or its components may be linked to numerous malignancies, including breast, colorectal, endometrial, and gastric cancer (8–11). MetS were also investigated as a potential PC risk factor. It was observed that in the general public, it was strongly linked to an elevated risk of developing PC (12). Previously, the number of MetS components and the probability of developing PC showed a strong correlation (13). The risk of PC varied among people with MetS, with the presence of four or five metabolic components being linked to the highest risk (14). However, a Japanese study found that only women with two or more metabolic components showed an elevated risk of PC (15). A subsequent prospective study, including over 580,000 people, also supported these findings (16). However, several shortcomings of these studies, including insufficient sample size, lack of ethnic/racial heterogeneity, and an inadequate assessment of confounders and/or reverse causality, resulted in contradictory findings.

Several studies have demonstrated that various aspects of MetS, such as obesity and type 2 diabetes, can increase the risk of PC (16–18). However, it is unclear which aspect of MetS is most strongly associated with PC and whether gender influences the effect of MetS on PC. The effects of MetS as a risk factor on PC were thoroughly reviewed and subjected to a meta-analysis. Furthermore, sub-analyses based on gender were conducted.

Methods and materials

Search strategy

PubMed, Embase, and the Cochrane Library databases were systematically searched for pertinent studies that were published between the creation of the database and November 1, 2022. The following search terms were used: ('pancreatic carcinoma' OR 'pancreatic cancer' OR 'pancreatic adenocarcinoma' OR 'pancreatic neoplasms') AND ('metabolic syndrome' OR 'Metabolic X Syndrome' OR 'Dysmetabolic Syndrome X' OR 'MetS'). Furthermore, the reference lists of qualified articles were visually examined for any additional pertinent studies.

Selection criteria

Based on the inclusion and exclusion criteria listed below, two researchers screened the retrieved publications independently, and discrepancies were settled by consensus. The following inclusion criteria were applied: (1) the publication that was written in English and was a cohort study or a case-control study; (2) data on the relative risk (RR), odds ratio (OR), or hazard ratio (HR) with a 95% confidence interval (95%CI) were available; (3) when multiple publications were produced from the same data, only the most comprehensive paper was selected.

Exclusion criteria for this study were as follows: (1) letters, case reports, reviews, expert opinions, or editorials were excluded; (2) excluded if they lacked critical data; (3) excluded if they failed to mention MetS and diagnostic criteria for PC explicitly; or (4) they were duplicates of other studies. Additionally, case-control studies were excluded from the meta-analysis but included in the systematic review.

Quality assessment

The Newcastle-Ottawa Scale (NOS) for quality evaluation of cohort studies and case-control studies was used to independently evaluate study quality (19). The NOS comprises eight components assigned to three groups based on selection, comparability, and research type exposure (case-control studies) or outcome (cohort studies). For each issue, a number of response alternatives were offered. A star system was employed to provide a semi-quantitative evaluation of the quality of the study. The highest-quality studies yielded a maximum of one star for each item, with the exception of the comparability item, which makes two stars. The NOS stars range between zero to nine. We discussed any disagreements until an agreement was reached. After examination, it was concluded that each study under investigation was of moderate to high quality.

Data extraction

The names of the first authors, the year the study was published, the country where the investigation was done, the duration of follow-up, the total number of patients, and the criteria for the definition of MetS were all retrieved separately by the two researchers for each study that was accessible. Using the most adjusted model, we derived the pooled risk estimates and associated 95%CIs. A discussion was used to settle any disagreements. To assess the effects of MetS components on the risk of developing PC, risk estimates were also gathered for each individual MetS component.

Statistical analyses

Using pertinent risk estimations, the relative risks (RR), hazard ratios (HRs), incident rate ratios, standardized incidence ratios (SIRs), and their 95%CIs were employed to evaluate the

relationship between MetS and PC risk. From the multivariable models of the original studies, adjusted risk estimates were generated. Additionally, we assessed how each component of the metabolic syndrome affected the risk of PC on an individual basis. Sensitivity analysis was also carried out to test whether any of the studies had shown a significant impact on the outcome. Using the random-effects model, the outcomes of the retrieved papers were combined. In order to evaluate the statistical heterogeneity across studies, the I^2 statistic was used. Low, moderate, and high levels of heterogeneity were estimated to be 25%, 50%, and 75%, respectively. To assess publication bias, the Egger test and funnel plotting were performed. When at least ten original publications were included, a P value < 0.05 showed publication bias. STATA (version 16.0) was used for conducting all analyses, and statistical significance was established at P< 0.05.

Results

Search results

Figure 1 displays a flow chart that illustrates the literature screening process. In total, 4,194 articles were retrieved from databases. Nine publications (12–15, 20–24), comprising two case-control studies (13, 23) and seven cohort studies (12, 14, 15, 20–22, 24), were considered in the systematic review. All the duplicate studies and those studies that failed to meet the inclusion criteria were eliminated. Meta-analysis was performed on all cohort studies (Figure 1).

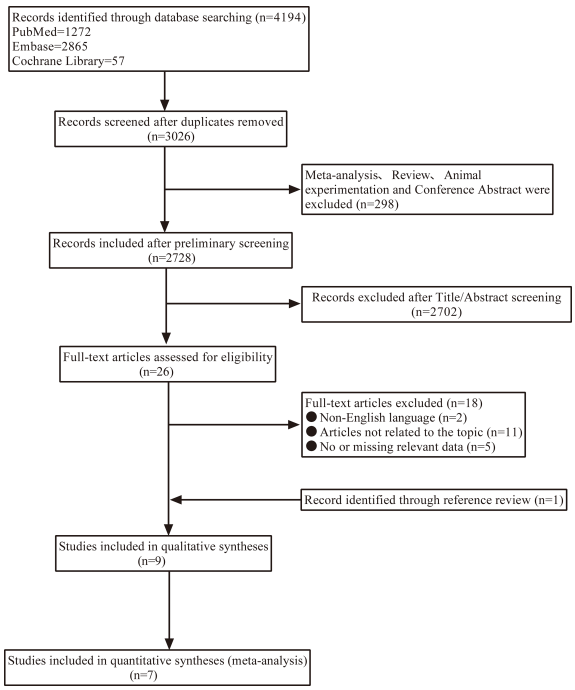

FIGURE 1
Flowchart for screening the literature.

Characteristics of included studies

A complete summary of the fundamental characteristics of each study that was included in this research is provided in Table 1. The study comprises research published between 2008 and 2022, and their quality scores, on average, were 7.2 stars. The median follow-up period per a study in the included literature ranged from 2.7 (Russo et al.) to 10.2 (Manami Inoue et al.) years. The adjusted analyses showed varied potential confounding factors (risk factors), including a maximum of 10 (21, 24) and a minimum of 5 confounders (15). In addition, only four studies reported an association between high blood glucose, blood pressure, triglyceride levels, and HDL-c levels with PC (12, 14, 22, 24). In comparison, five studies reported an association of obesity with PC (12, 14, 21, 22, 24).

Meta-analysis results

Figures 2–8 show forest plots for the PC and MetS meta-analysis. In comparison with non-MetS individuals, patients having MetS had a greater probability of getting PC (RR 1.34, 95%CI 1.23–1.46, P<0.0001, I^2 = 38.8%) (Figure 2). For the subgroup analysis of the prevalence of PC in MetS patients, the study population was divided into male and female groups. It was observed that the prevalence of PC was remarkably higher in males and females with MetS than among non-MetS patients. Among MetS patients, females were more likely to develop PC than males (male: RR 1.26, 95%CI 1.03–1.54, P=0.022; females: RR 1.64, 95%CI 1.41–1.90, P< 0.001) (Figure 3).

Table 2 summarizes the diagnostic criteria for every single component of MetS present in each study, and Table 3 lists the numerous types of diagnostic criteria for MetS. These findings demonstrated that the risk of PC was not correlated with obesity or hypertriglyceridemia (obesity: RR 1.13, 95%CI 0.96–1.32, P=0.151; hypertriglyceridemia: RR 0.96, 95%CI 0.87–1.07,P=0.486) (Figures 4, 5).While hyperglycemia, hypertension and low HDL-c increased the risk of PC (hyperglycemia: RR 1.55,95%CI 1.42–1.70, P< 0.001; hypertension: RR 1.10, 95%CI 1.01–1.19, P=0.027; low HDL-c: RR 1.24, 95%CI 1.11–1.38, P< 0.001) (Figures 6–8). The results of the sensitivity analysis showed that the link between MetS and the risk of PC was unaffected noticeably due to the lack of any studies (Figure 9).

Discussion

Among the components of MetS, dyslipidemia, hypertension, diabetes, and obesity-related biological processes are closely related to one another and increase the risk of developing numerous diseases. The strongest risk factor for PC is diabetes, which is one of the various components that constitute MetS (13). According to UK Biobank data, the PC risk was increased in people with MetS (HR = 1.31, 95%CI 1.09–1.56), hyperglycemia (HR = 1.60, 95%CI 1.31–1.97), and abdominal obesity (HR = 1.24, 95%CI 1.02–1.50). However, these two last MetS components (central obesity and

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TABLE 1 Characteristics of the studies included in the quantitative and qualitative review.

Author	Year	Country	Study Type	Age (range or mean)	MetS criteria	Follow-up	Sample size	No. of cases	Quality assessment
Antonio Russo (20)	2008	Italy	Cohort	≥40	Pharmacological definition	median follow-up 2.7 years	16,677	43	6
Manami Inoue (15)	2009	Japan	Cohort	M:56.5 ± 8.2 F:55.5 ± 8.1	AHA	average follow-up 10.2 years	27,724	65	6
Valentina Rosato (13).	2011	Italy	Case control	34-80	AHA	17years	978	21	6
Bin Xia (12).	2020	China	Cohort	MetS (+):58.1 MetS (-):55.8	IDF	MetS (+):6.5 years (1.3) MetS (-):6.6 years (1.2)	475,078	565	8
Sung Keun Park (14)	2020	South Korea	Cohort	MetS (+):60.3 ± 9.1 MetS (-):56.92 ± 8.4	IDF	4years	222,838	381	8
HyeSoo Chung (21)	2021	South Korea	Cohort	MetS (+):60 ± 9 MetS(-):59.3 ± 8.7	IDF	median follow-up 6.1 years	347,434	886	7
Joo-Hyun Park (22)	2022	South Korea	Cohort	48.9	IDF	median follow-up 5.1 years	8,203,492	8010	8
Joseph A (24)	2022	French	Cohort	MetS (+):58.40 ± 7.61 MetS(-):55.48 ± 8.15	NCEP-ATPIII	median follow-up 7.1 years	366,494	478	8
Tomàs López-Jiménez (23)	2022	Spain	Case control	≥40	AHA	11years	183,284	1996	8

AHA, American Heart Association;
IDF, International Diabetes Federation;
NCEP-ATP III, National Cholesterol Education Program Adult Treatment Panel III.

hyperglycemia) seem to exhibit an independent connection in increasing the risk of PC (12).

The present study indicated a correlation between MetS and the risk of PC. The hypothesis of this study was supported by the two case-control studies that were part of the systematic review. Low degrees of study heterogeneity were observed, however. Through subgroup analyses, the cause of heterogeneity was identified, and we came to the conclusion that among MetS patients, the risk of developing PC was higher in women than in men. This observation was consistent with the findings of one of the previous studies (25).

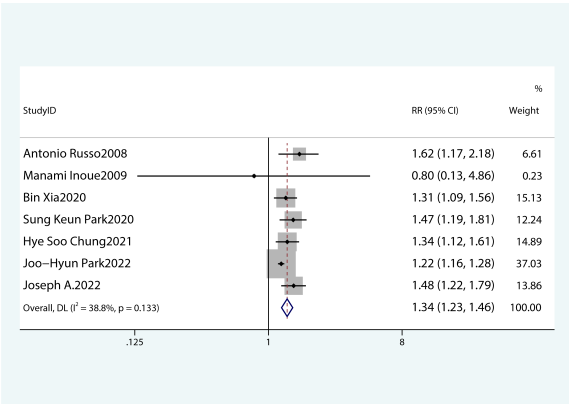

FIGURE 2
Meta-analysis of studies on the correlation of metabolic syndrome with pancreatic cancer;.

Moreover, a summary of each MetS component's impact on PC risk was produced. According to the majority of studies (26, 27), PC risk is correlated with hypertension, hyperglycemia, low HDL-c levels, and particularly with hyperglycemia.

There has been extensive research on the pathogenesis of PC in diabetes mellitus or hyperglycemia. PC cells multiplied and invaded as a result of p38 MAPK elicited by high glucose levels. Additionally, P38 MAPK was also activated as a result of cellular stress and inflammatory conditions, which could control metastasis, apoptosis, and cell proliferation. PC cell proliferation and development occurred as a result of heightened paracrine effects of inflammatory cytokines (such as IL-6) and VEGF, which were mediated by P38 MAPK. Moreover, elevated hyperglycemia *via RET* (a proto-oncogene that encodes a receptor tyrosine kinase for members of the glial cell line-derived neurotrophic factor family of extracellular signaling molecules) can boost PC cell invasion and proliferation (18). Meta-analyses had also shown that dietary cholesterol might be linked to a higher risk of PC (28), which was

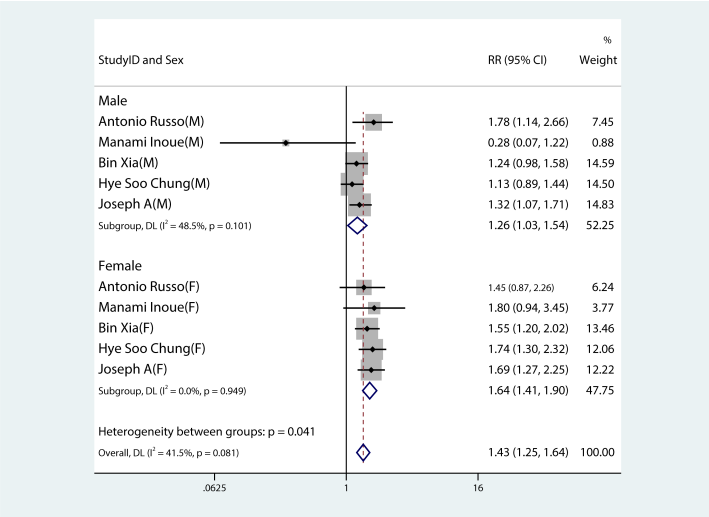

FIGURE 3
Forest plot demonstrating the association between the metabolic syndrome and the risk of developing pancreatic cancer in both males and females.

confirmed by the results of this study. Surprisingly, obesity and hypertriglyceridemia were not associated with PC in the meta-analysis. Previous studies also revealed that there is an increased risk of developing cancer due to obesity (17, 29–31), contradictory to the outcomes of this study. Evidence suggested that the development and progression of PC were caused by an increase in various hormones in obese people, including insulin, adipokines, and resistin (18). Resistin is an adipocyte-secreting hormone involved in insulin resistance and inflammation. It has the ability to affect the progression of the PC. In patients with pancreatic ductal adenocarcinoma, it was considered a negative independent prognostic factor for relapse-free survival (32). Therefore, we speculate that the possible reason for this is insulin resistance and/or low HDL-c levels in most obese individuals, which can increase the cancer risk. Moreover, as per the outcomes of a meta-analysis performed in 2012, the body mass index and central obesity are linked to an average RR of 1.10 for a five-unit rise in the occurrence of PC (33). This correlation applies to African Americans (34) but not to residents of Lithuania (35) or Singapore's Chinese nonsmoking population (36). Asians comprised the majority of the ethnicities examined in the studies used in the meta-analyses conducted in this research. The European Australasian (RR: 1.18, 95%CI 1.09–1.27) and North American (RR: 1.07, 95%CI 1.03–1.11) populations, however, showed favorable relationships between MetS and PC (37). These outcomes can be explained based on different study methodologies and variations, for example, socioeconomic, genotypic, and environmental aspects of these diverse groups.

Elevated triglyceride levels and reduced HDL-c are the components of MetS. Previous studies on dyslipidemia and the risk of PC produced controversial results (36, 38, 39). In the present study, no evidence of increased risk of developing PC due to high triglyceride levels was obtained.

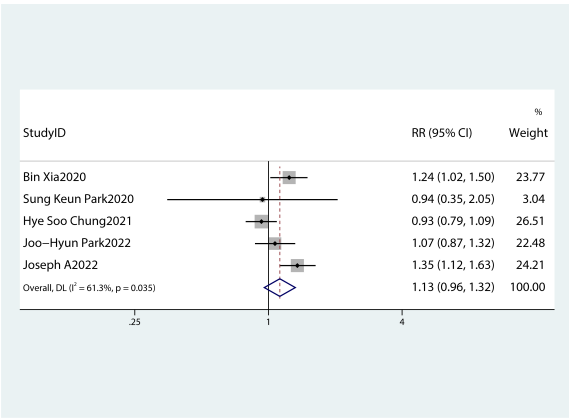

FIGURE 4
A forest plot demonstrating the relationship between obesity and the risk of pancreatic cancer.

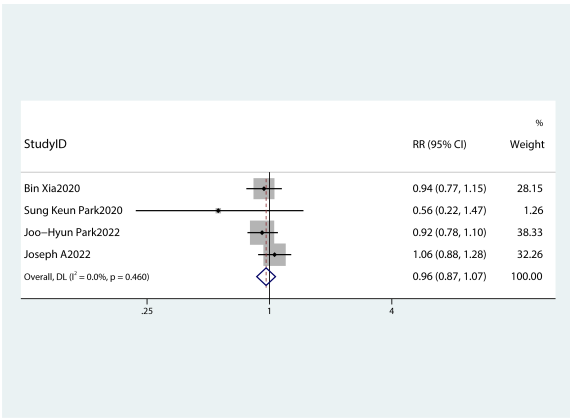

FIGURE 5
A forest plot demonstrating the relationship between hypertriglyceridemia and the risk of pancreatic cancer.

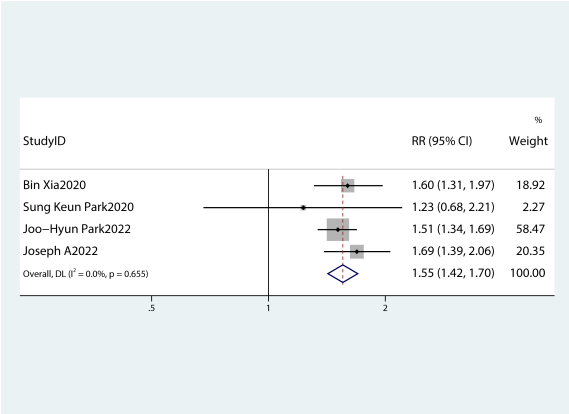

FIGURE 6
A forest plot demonstrating the relationship between hyperglycemia and the risk of pancreatic cancer.

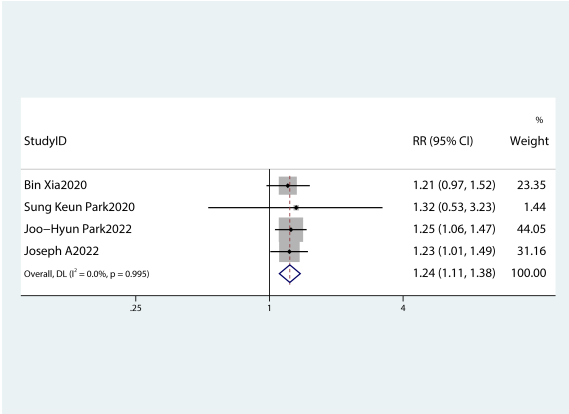

FIGURE 8
A forest plot demonstrating the relationship between low HDL-c levels and the risk of pancreatic cancer.

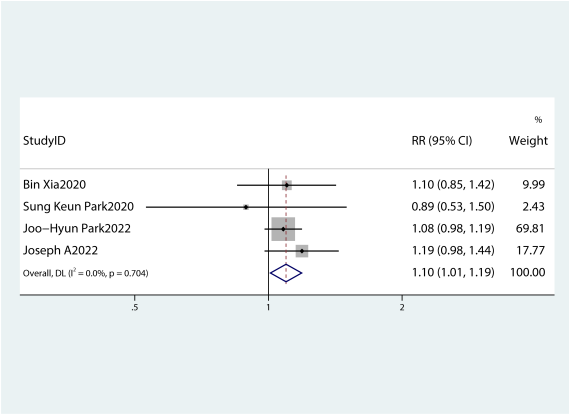

FIGURE 7
A forest plot demonstrating the relationship between hypertension and the risk of pancreatic cancer.

MetS is reversible. In patients with MetS, and the lifestyle-modification intervention was successful. It resulted in easing the condition and decreasing the severity of associated abnormalities (triglycerides, waist size, systolic and diastolic blood pressure, and fasting blood glucose) (39). Previous results also suggested that MetS could be a risk factor for PC that is modifiable (22). The connection between MetS and the risk of PC may be explained by various molecular pathways. First, insulin resistance is a significant contributor to the pathophysiology of MetS. Elevated insulin levels, as well as modulation of insulin-like growth factors-1 and -2, may contribute to PC by boosting cell proliferation and angiogenesis while inhibiting cell death (40–42). Moreover, visceral adipose tissue has a high metabolic rate and secretes a variety of cytokines that promote inflammation (41, 42). Chroniclow-grade inflammation, including these cytokines, may increase the risk of PC by increased production of reactive oxygen species and cell cycle rates, thus attenuating tumor suppressor activity (42, 43). Finally, MetS have been linked to the altered composition of gut microbiota, decreased microbial diversity, and decreased gene richness, all of which are crucial for

TABLE 2 Diagnostic criteria for any single component of metabolic syndrome in each study.

Author	Year	hypertension	hyperglycemia	obesity	hypertriglyceridemia	Low HDL-c
Antonio Russo (20)	2008	Use of drugs for hypertension	Use of drugs for diabetes	-	-	Use of drugs for hypercholesterolemia
Manami Inoue (15)	2009	BP≥130/85 mmHg and/or use of antihypertensive agents	glucose ≥ 5.55 mmol/l (100 mg/dl) fasting or ≥ 7.77 mmol/l (140 mg/dl) non-fasting	BMI ≥ 25 kg/m^2	high serum triglycerides ≥ 1.69 mmol/l (150 mg/dl)	low HDL-c< 1.03 mmol/l (40 mg/dl) for men and <1.29 mmol/l (50 mg/dl) for women
Bin Xia (12)	2020	systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg or treatment of previously diagnosed hypertension	FPG ≥ 100 mg/dLor previously diagnosed type 2 diabetes	BMI > 30 kg/m^2	TGlevels ≥ 0.7 mmol/L (150 mg/dL) or currently on medications for hypertriglyceridaemia	HDL-c< 0.9 mmol/L (40 mg/dL) for men and < 1.29 mmol/L (50 mg/dL) in women or specific treatment for previously detected reduced HDL -c.

(Continued)

TABLE 2 Continued

Author	Year	hypertension	hyperglycemia	obesity	hypertriglyceridemia	Low HDL-c
Sung Keun Park (14)	2020	BP ≥130/85 mm Hg	FPG ≥ 100 mg/dL	WC≥90 cm in men and ≥85 cm in women	TG levels ≥150 mg/dL	HDL-c< 40 mg/dL for men and < 50 mg/dL for women
Hye Soo Chung (21)	2021	BP ≥130/85 mmHg or the use of antihypertensive agents	FPG≥5.6 mmol/L (100 mg/dL) or useofan antidiabetic drug	BMI is ≥25 kg/m^2	serum triglyceride levels ≥1.7 mmol/L (≥150 mg/dL) or the current use of lipid-lowering agents	HDL-c <1.0 mmol/L (40 mg/dL) in men or<1.3 mmol/L (50 mg/dL) in women or the current use of lipid-lowering agents
Joo-Hyun Park (22)	2022	systolic ≥130 or diastolic ≥80 mmHg or the use of antihypertensive agents	FPG≥100 mg/dL or the use of an antidiabetic drug	WC≥90 cm in men and ≥85 cm in women	TG levels ≥150 mg/dL or the use of a relevant drug	HDL-C <40 mg/dL for men and <50 mg/dL for womenor the use of a relevant drug
JosephA (24)	2022	systolic ≥ 130 mmHg and diastolic ≥ 85 mmHg, or previously diagnosed high BP, or regular use of BP-lowering medication.	HbA1c≥ 5.7%, regardless of diabetes status.	WC≥102 cm in men or ≥88 cmin women	triglycerides were considered elevated if measured at ≥1.7 mmol/L	reduced HDL was defined as ≤1.03 mmol/L in men and ≤1.29 mmol/L in women, or regular use of cholesterol-lowering medication

BP, blood pressure; FPG, fasting plasma glucose; BMI, body mass index; WC, waist circumference; TG, plasma triglyceride; HDL-c, high-density lipoprotein cholesterol.
-, It means that the diagnostic criteria for this component of metabolic syndrome are notprovided in the article.

TABLE 3 Different Criteria for MetS Diagnosis.

MetS Diagnosis Criterion	Details
Pharmacological definition	Patients who are also taking medicine for high cholesterol, high blood pressure, and diabetes
NCEP-ATP III	(1) WC≥102 cm in men and ≥88 cmin women; (2) TG≥1.7 mmol/L; (3) HDL-c ≤ 1.03mmol/L in men and ≤1.29 mmol/L in women (4) BP≥130/85 mmHg; (5) FPG≥6.1 mmol/L; ≥3 above components can be diagnosed as MetS.
IDF	(1) central obesity(WC ≥ 90 cm and ≥ 80 cm in Asians, with other values for other ethnicities; or BMI > 30 kg/m2); (2) TGlevels ≥ 0.7 mmol/L (150 mg/dL); (3) HDL-c< 0.9 mmol/L (40 mg/dL) for men and < 1.29 mmol/L (50 mg/dL) in women or specific treatment for previously detected reduced HDL -c; (4) systolic ≥ 130 mmHg or diastolic ≥ 85 mmHg or treatment of previously diagnosed hypertension; (5) FPG ≥ 100 mg/dLor previously diagnosed type 2 diabetes; central obesity plus any two of the above four factors can be diagnosed as MetS.
AHA	(1) FPG ≥ 100 mg/dL or receiving drug therapy for hyperglycemia; (2) BP ≥ 130/85 mmHg or receiving drug therapy for hypertension; (3) TG ≥ 150 mg/dL or receiving drug therapy for hypertriglyceridemia; (4) HDL-c < 40 mg/dL in men or < 50 mg/dL in women or receiving drug therapy for reduced HDL-C; (5) WC≥90 cm in men or ≥80 cmin women; ≥3 above components can be diagnosed as MetS.

NCEP-ATP III, National Cholesterol Education Program Adult TreatmentPanel III;
IDF, International Diabetes Federation;
AHA, American Heart Association;
BP, blood pressure;
FPG, fasting plasma glucose;
BMI, body mass index;
WC, waist circumference;
TG, plasma triglyceride;
HDL-c, high-density lipoprotein cholesterol.

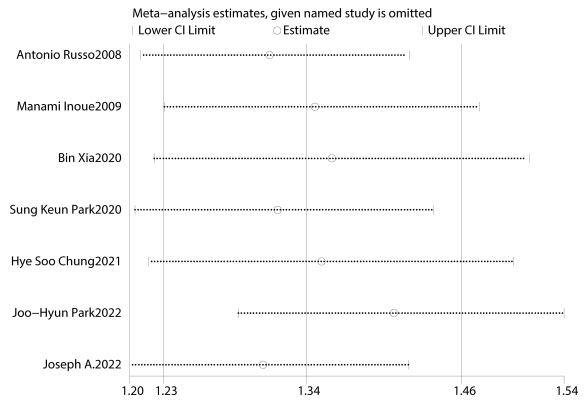

FIGURE 9
Sensitivity analyses for pancreatic cancer;.

carcinogenesis and tumorigenesis (43, 44). Therefore, it can be concluded that preventing or recovering from MetS might reduce the risk of developing PC.

However, this meta-analysis has some limitations. First, like any other meta-analysis, residual confounding from the original studies cannot be eliminated. After correcting for the majority of significant confounding factors, residual or unknown confounders may persist. Because each trial was adjusted for a unique set of variables, meta-analyses may have been heterogeneous. Second, the comprehensiveness of this study was limited by the relatively small number of pertinent publications, which precluded analyses for other relevant characteristics, including age and ethnicity. Third, the metabolic components were not directly assessed using the same technique, which may result in high heterogeneity between studies. However, the sensitivity analysis and subgroup analysis showed the robustness of our outcomes.

In conclusion, our meta-analysis revealed that MetS showed a remarkable correlation with a high risk of developing PC in both genders, with a higher risk in females as compared to males. Low HDL-c levels or hyperglycemia may be primarily responsible for the higher risk of PC in individuals with MetS. However, obesity and hypertriglyceridemia do not increase the risk of PC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Author contributions

All authors contributed to the study's conception and design. LZ was in charge of material preparation, data collection, and analysis. SL wrote the first draft of the paper. JFL contributed to the writing, revision, and review of the manuscript. GT reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1116582/full#supplementary-material

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Obesity, non-alcoholic fatty liver disease and hepatocellular carcinoma: current status and therapeutic targets

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Obesity is a global epidemic and overwhelming evidence indicates that it is a risk factor for numerous cancers, including hepatocellular carcinoma (HCC), the third leading cause of cancer-related deaths worldwide. Obesity-associated hepatic tumorigenesis develops from nonalcoholic fatty liver disease (NAFLD), progressing to nonalcoholic steatohepatitis (NASH), cirrhosis and ultimately to HCC. The rising incidence of obesity is resulting in an increased prevalence of NAFLD and NASH, and subsequently HCC. Obesity represents an increasingly important underlying etiology of HCC, in particular as the other leading causes of HCC such as hepatitis infection, are declining due to effective treatments and vaccines. In this review, we provide a comprehensive overview of the molecular mechanisms and cellular signaling pathways involved in the pathogenesis of obesity-associated HCC. We summarize the preclinical experimental animal models available to study the features of NAFLD/NASH/HCC, and the non-invasive methods to diagnose NAFLD, NASH and early-stage HCC. Finally, since HCC is an aggressive tumor with a 5-year survival of less than 20%, we will also discuss novel therapeutic targets for obesity-associated HCC and ongoing clinical trials.

KEYWORDS

hepatocellular carcinoma (HCC), obesity, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), therapeutic targets, animal models, metabolic dysfunction-associated fatty liver disease (MAFLD)

1 Introduction

Liver cancer is the sixth most frequently diagnosed cancer worldwide and has the third highest fatality rate of all cancers, with a 5-year survival of less than 20% (1, 2). The incidence of liver cancer is rising continuously and globally its mortality is expected to increase by 41% by 2040 (3, 4). Hepatocellular carcinoma (HCC), the most common form of liver cancer, accounts for over 90% of the cases (5). The occurrence of HCC is attributed to hepatitis B (HBV), hepatitis C (HCV), alcohol abuse, aflatoxin B1, iron accumulation, obesity and diabetes mellitus

(6). Over the past decade, HBV and HCV infection are the primary risk factors for HCC, constituting 80% of HCC cases globally (7, 8). With the widespread availability of effective vaccination and antiviral therapies for HBV and HCV infection, the rates of viral-associated HCC are expected to decline in the coming years (9, 10).

Given the worldwide obesity pandemic, a growing amount of evidence suggests that obesity and the accompanying development of non-alcoholic fatty liver disease (NAFLD) and its aggressive form non-alcoholic steatohepatitis (NASH) are becoming the leading contributing factors to the rising incidence of HCC (11–13). Notably, efforts are underway to rename NAFLD as metabolic dysfunction-associated fatty liver disease (MAFLD), which emphasizes the role of the metabolic syndrome, obesity and Type 2 diabetes mellitus (T2DM) in contributing to the burden of liver disease (14). Obesity is a major driver of NAFLD and NASH, around 50% of NAFLD patients and 80% of NASH patients present with obesity (15). Notably, obesity itself is an independent risk factor for the onset and development of HCC. Obesity is associated with a 2-3 fold increased risk of HCC (16), and obese individuals exhibit an approximately 4-fold increase in HCC-related mortality and 2-fold increase in life-threatening complications following surgical cancer treatments (17–19). Obesity usually causes a diversity of complications, including cardiovascular diseases, insulin resistance (IR), T2DM, hypertension and hyperlipidemia (20–22). Indeed, IR and T2DM are also independent risk factors for chronic liver disease and HCC (23). The incidence of HCC among those with T2DM increased by 2 to 3-fold in different cohort studies (24, 25). In the context of HBV or HCV infection, the strong synergy between obesity and diabetes conveys more than a 100-fold HCC risk (26).

In principle, patients with HCC are stratified and allocated to treatment based on tumor stage, liver function and performance status (27). Resection, transplantation and local ablation are the first choices for patients with early-stage HCC tumors, while transarterial chemoembolization (TACE) is the first-line treatment for patients at intermediate stage and those with advanced stages will first receive systemic therapies (28). However, on average, patients are older and are more frequently diagnosed at advanced stages (29). Currently, systemic therapies including immune checkpoint inhibitors (ICIs), tyrosine kinase inhibitors (TKIs) and monoclonal antibodies are now improving the prognosis of HCC patients (28). However, ICIs may not be as effective for NASH-HCC as they are more appropriate for viral HCC. International clinical practice guidelines for HCC do not consider etiology, as there is insufficient data to draw specific conclusions or recommend etiology-specific treatment for patients with HCC (29). Furthermore, although the growing prevalence of obesity-associated HCC and vast studies are progressing, currently, there are no FDA-approved drugs and treatments for NASH yet. The therapeutic options for obesity-associated HCC are an unmet clinical need. A better understanding of obesity-associated HCC will help to establish more effective treatment strategies. Herein, this review discusses the underlying pathological mechanisms and signaling pathways of obesity leading to HCC. In addition, we summarize the novel potential therapeutic targets and ongoing clinical trials in HCC patients with obesity (Figure 1).

2 Pathophysiological mechanisms of obesity-associated HCC

2.1 Insulin resistance and hyperinsulinemia

Insulin resistance (IR) and subsequent hyperinsulinemia are major pathological consequences of obesity, which significantly contribute to the development of NAFLD, NASH and HCC (30, 31). Insulin is a key

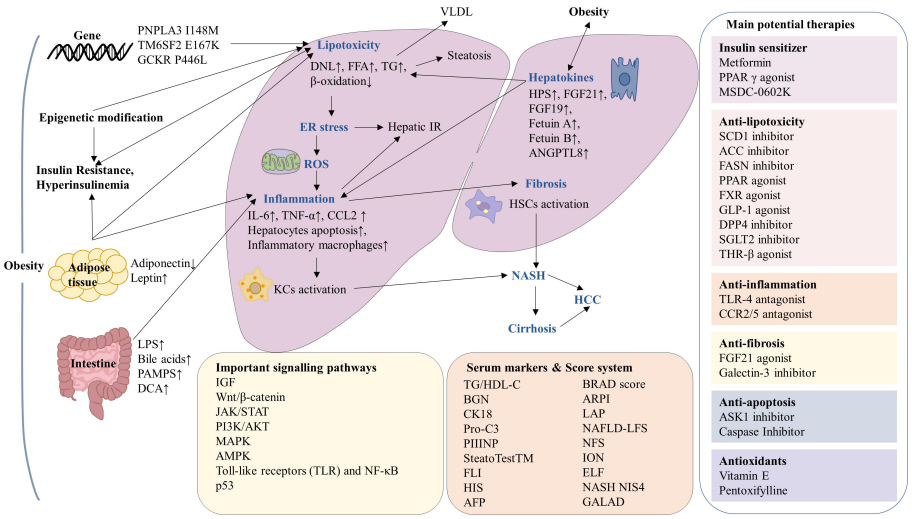

FIGURE 1
Summary of the mechanisms and serum markers of obesity-associated hepatocellular carcinoma (HCC) and current potential therapies. In the context of obesity, there is an increased risk of insulin resistant, gene variation, adipose tissue disfunction, epigenetic modification and intestinal microbiota imbalance, leading to lipotoxicity, endoplasmic reticulum (ER) stress, reactive oxygen species (ROS), inflammation and fibrosis, which ultimately progression to HCC. The molecular mechanisms involved in obesity-associated HCC including IGF, Wnt/β-catenin, JAK/STAT, PI3K/AKT, MAPK, AMPK, TLR, NF-κB and p53 signaling pathways. The serum markers and score system have been used in the diagnosis of NAFLD and NASH. Current potential therapies for obesity-associated HCC can be classified as insulin sensitizers, antioxidants as well as drugs against lipotoxicity, inflammation, fibrosis and apoptosis.

regulator of glucose metabolism and an increase in hepatic IR impairs glucose homeostasis by enhancing hepatic gluconeogenesis and glycogenolysis, leading to glucose intolerance (32, 33). Glucotoxicity is associated with elevated glucose levels and further contributes to IR (34). Both IR and hyperinsulinemia increase the serum level of insulin-like growth factor 1 (IGF-1) and the biological activity of IGF-1. IR and the binding of IGF-1 to insulin-like growth factor 1 receptor (IGF-1R) will trigger their downstream cellular pathways, such as phosphatidylinositol-3 kinase (PI3K), protein kinase B (AKT) and mitogen-activated protein kinase (MAPK), which induce HCC cells to proliferate and inhibit apoptosis, ultimately promoting the tumorigenesis of HCC (35, 36).

IR leads to a diverse range of metabolic and molecular effects including inflammation, endoplasmic reticulum (ER) stress and oxidative stress resulting in DNA damage which together contribute to hepatic cell injury and ultimately carcinogenesis in NASH (33, 37). Excessive lipid accumulation in liver is an important consequence of IR and the imbalanced energy metabolism leads to hepatic lipotoxicity and an increased release of free fatty acids (FFAs) in the serum and liver (38–40), with the deposition of large amounts of triglyceride (TG) in the liver which accelerates hepatocyte degeneration, fatty liver disease and fibrosis (41, 42). In hepatocytes, IR also causes steatosis through alterations in lipoprotein metabolism (33).

2.2 Lipid accumulation and lipotoxicity

The "lipid-rich condition" is highly characteristic of obesity-associated HCC and the deregulated hepatic lipid metabolism has been considered a driving force of HCC (43, 44). Hepatic lipid accumulation results from excessive lipid influx or impaired lipid export. Lipid accumulation includes four separate mechanisms 1) increased hepatic uptake of circulating fatty acids, 2), increased hepatic *de novo* lipogenesis (DNL), 3) decreased hepatic β-oxidation and 4) decreased hepatic lipid export. In obese individuals, the elevations of plasma FFA derived from adipose tissue depots and hepatic DNL promotes hepatic lipid accumulation, while hepatic β-oxidation and lipid secretion in very low-density lipoproteins (VLDL) decrease hepatic lipid content (45). Ectopic lipid accumulation in the liver is directly related to hepatic lipotoxicity, leading to exacerbation of steatosis and HCC development (46, 47).

Lipotoxicity is generally defined as the dysregulation of lipid environment and/or intracellular lipid composition, leading to an increased concentration of harmful lipids, impairing cellular homeostasis and disrupting tissue function (48, 49). Lipotoxicity ultimately leads to cell injury and chronic inflammation, followed by progression from NAFLD to NASH (50). Well-documented evidence indicates that the risk for lipotoxicity is also conveyed by FFAs rather than TG (51), suggesting that several underlying mechanisms that contribute to hepatocarcinogenesis. Lipotoxicity is a consequence of aberrant lipid metabolism. Hepatic metabolism of FFAs induces the formation of toxic metabolites, and they are responsible for inflammation, oxidative stress (OS) and liver parenchyma injury (51). The elevated FFA in hepatocytes promotes mitochondrial β-oxidation, which causes mitochondrial dysfunction and increased oxidative stress and leading to steatosis (52). FFAs have an additional function as signaling molecules, an energy source and structural components of the cell membrane, all of which are essential for cancer cell proliferation (43). FFAs are able to interfere with cellular signaling mechanisms and regulate gene transcription, activating various oncogenic pathways (53, 54). The overexposure of FFAs promote the expression of pro-inflammatory cytokines, impair insulin signaling and enhance apoptosis of hepatocyte in the context of ER and oxidative stress (51). In addition to direct cytotoxic effects, the accumulation of FFAs aggravates IR and hyperinsulinemia (55), which leads to further hepatic lipid accumulation (56), promotes inflammation (57) and increases carcinogenic fibrogenic responses (58) as well as mitogenic responses (56).

2.3 Adipose tissue and adipokines

Adipose tissue (AT) plays a major role in whole-body energy balance, as it responds rapidly and dynamically to changes in nutrient deprivation and excess through adipocyte hypertrophy and hyperplasia (59). AT expansion and progressive AT dysfunction is a key event in the development of obesity-associated HCC, due to the existence of adipose tissue-liver crosstalk (46, 60). AT remodeling is a continuous process that is pathologically accelerated in the obese state, and is characterized by a reduction in angiogenic remodeling, an overproduction of extracellular matrix (ECM), a heightened state of immune cell infiltration and subsequent pro-inflammatory response in obese individuals (59). AT is major locus of inflammation in obesity-related HCC (52, 61), and proinflammatory cytokine levels are markedly elevated in the AT of obese individuals. The accumulation of inflammatory cells, especially AT resident macrophages, in visceral AT, is a hallmark of AT dysfunction (62). The activation of inflammation and the recruitment of macrophages in visceral AT and subcutaneous AT of NAFLD patients correlates with the progression from simple steatosis to NASH and fibrosis (61, 63).

AT is an important energy storage organ and a key endocrine organ with active metabolism (64). The hormones (leptin, adiponectin), cytokines (tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), interleukin 6 (IL-6), and interleukin-8 (IL-8)), chemokines (Chemokine C-C motif ligand 2 (CCL2)), extracellular matrix proteins (matrix metallopeptidase 2 (MMP2) and matrix metallopeptidase 9 (MMP9)) and angiogenic proteins (vascular endothelial-derived growth factor (VEGF) are secreted from AT and collectively known as adipokines (65). Excess production of storage lipids leads to imbalanced adipokine secretion (adiponectin, leptin) (47, 66) that may profoundly affect not only the local AT itself but also the liver. The enhanced production of inflammatory chemokines and cytokines (TNF-α, IL-6) and reduced beneficial ones (adiponectin) contributes to acute and chronic inflammation as well as peripheral and hepatic IR (51). The expansion of AT, independent of other concomitant factors, deprives NAFLD patients of the anti-inflammatory and anti-fibrotic

effects of adiponectin (65). In obese patients, increased leptin and decreased adiponectin level may lead to hepatic steatosis and activate inflammation and fibrosis (63).

2.4 Endoplasmic reticulum stress

Endoplasmic reticulum (ER) dysfunction is a common phenomenon in obesity-related HCC (67). ER stress is thought to drive adiposity by reducing energy expenditure (68) and emerging data suggest ER stress plays an important role in the progression of obesity, hepatic steatosis, NASH and HCC (69, 70).

An excessive influx of fatty acid leads to severe ER stress in obese states. In turn, ER enhances lipogenesis and hepatic steatosis (70). As a result, there is a positive feedback on ER stress and hepatic steatosis, which exacerbates liver damage (71). In addition, there is evidence that obesity-induced ER stress and inflammation in the liver can lead to hepatic IR (72). ER regulates protein synthesis and folding for various cellular processes. For instance, ER stress can induce hepatocyte apoptosis by activating C/EBP homologous protein and c-Jun amino-terminal kinases (JNK) signaling (48). ER stress initiates the unfolded protein response (UPR) to restore ER proteostasis, while UPR can cause inflammation and influence the development and aggressiveness of HCC (73). Oxidative stress is often accompanied by ER dysfunction. ER stress increases the production of reactive oxygen species (ROS) in hepatocytes, causing oxidative stress and subsequent genomic instability. In addition, ER and oxidative stress stimulate the sensitivity of hepatocytes to lipotoxic death, thereby releasing inflammatory mediators and inducing hepatic malignancy (74).

2.5 Oxidative stress

Oxidative stress is characterized by excessive levels of ROS. It is considered a tumor promoter by contributing to the initiation and progression of obesity-associated HCC (75, 76). The mitochondrial respiratory chain is the main source of hepatic oxidative stress derived from energy metabolism (77). In addition, other factors contributing to oxidative stress in obesity are: fatty acid accumulation, ER stress, chronic inflammation, abnormal postprandial ROS production, hyperleptinemia, tissue dysfunction and low antioxidant capacity (76).

Oxidative stress is not only a consequence but also a trigger of obesity and it plays a causative role in obesity development by stimulating white adipose deposition and increasing adipocyte proliferation (78). Hepatocyte exposure to excess ROS results in hepatocyte apoptosis and eventual cell death (79). Oxidative stress is closely linked to inflammation in obesity. Oxidative stress triggers the release of pro-inflammatory cytokines (TNF-α) and activates the inflammatory transcription factors (nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1)), thus leading to advanced fibrosis and cirrhosis, raising the risk of HCC (75, 76). Furthermore, the proinflammatory cytokines in turn enhance ROS production and cellular injury (75). Additionally, oxidative stress contributes to the release of pro-fibrogenic factors, which are involved in the initiation of fibrosis in HCC (79). Oxidative stress directly causes DNA alterations which leads to genomic instability and mutations in proto-oncogenes and tumor suppressor genes, thereby promoting tumor transformation (31).

2.6 Imbalance in intestinal microbiota

Intestinal microbiota plays an integral role in maintaining physiological, metabolic and enzymatic homeostasis (80). Sedentary lifestyle and high intake of a diet rich in saturated fat, sucrose and fructose have led to gut microbiota dysbiosis. Growing evidence has elucidated the association of gut microbiota dysbiosis with obesity, NAFLD and NASH (81–83), and it is a driving force in the progression of NAFLD and NAFLD-HCC through the gut-liver axis (84, 85).

Patients with NAFLD and NASH show significantly increased intestinal and detectable lipopolysaccharides (LPS) in portal blood. Alteration in intestinal microflora triggers inflammation, immune response, and immune cell infiltration of liver and AT (86). LPS is able to augment TNF-α production and activate the toll-like receptor 4 (TLR-4) pathway, thereby inducing a hepatic inflammatory response, leading to the progression of liver fibrosis and HCC (87, 88). In gut microbiota dysbiosis, pathogen-associated molecular patterns (PAMPs) are released. They are recognized by TLRs and potentiate innate immune responses (86). Bile acids are an important metabolite that links the gut microbiome with liver diseases (88). Dysregulated bile acid-microbiome crosstalk induces inflammation and HCC progression. Dysbiosis of the gut microbiome in obesity and NASH may induce the secretion of deoxycholic acid (DCA), a secondary bile acid that induces DNA damage. The high level of DCA in liver promotes the secretion of various inflammatory and tumor-promoting factors, thus further contributing to the development of HCC (89). Through the modulation of the gut-liver axis, the gut microbiota dysbiosis causes increased intestinal permeability, transfer of LPS, unrestricted transfer of microbial metabolites to the liver, immune activation and altered bile acid signaling, all of which contribute to liver inflammation, fibrosis, and eventually proceed to HCC (81, 90), thus further confirming the crucial role of the gut-liver axis in the pathogenesis of HCC.

2.7 Inflammation and Immune response

Obesity is characterized by a low-grade chronic inflammation which is a pivotal component for HCC development in the context of obesity (46). In obese individuals, the inflammation is attributed to the exacerbation of inflammatory cytokines deriving from extrahepatic sites (e.g. AT expansion and intestinal inflammation), or within the liver, the activation of Kupffer cells (KCs) and lipotoxicity of hepatocytes (51, 91).

The pathological cascade associated with inflammation leads to the activation of hepatic stellate cells (HSCs) and their fibrogenic differentiation, ultimately leading to liver fibrosis and cirrhosis (92).

Chronic inflammation facilitates the massive release of proinflammatory cytokines (IL-6, TNF-α) (66). Through activity on multiple oncogenic molecular pathways (e.g. inhibitor of kappa kinase IKK/c-Jun amino-terminal kinases JNK, signal transducer and activator of transcription STAT and NF-κB pathways), high levels of cytokines trigger IR, oxidative stress, lipotoxicity, hepatocyte cell death, liver inflammation, fibrosis, and pathological angiogenesis, thus promoting the progression from simple obesity-related hepatic steatosis to HCC (26, 80, 93).

Obesity modulates intrahepatic immunity and induces a microenvironment of immune intolerance, which leads to the progression of HCC (94). In the setting of obesity, pro-inflammatory cytokines, lipotoxicity and intestinal flora affect the activation of innate and adaptive immunity by stimulating liver-resident macrophages, named KCs, and recruitment of inflammatory macrophages to the liver (94–96). Hepatocytes and KCs secrete chemokines, including CCL2, thereby increasing the liver macrophage pool through monocyte infiltration (97). The recruitment of these immune cells to the liver is an important step in the pathogenesis of NASH and HCC (98). Activation of innate immunity drives further hepatic infiltration and accumulation of inflammatory cells, thereby aggravating inflammation and damage to the liver, regulating the progression of liver fibrosis, angiogenesis and carcinogenesis (95). Intrahepatic activated $CD8^+$ T cells and natural killer T (NKT) cells are increased in NASH (99). NKT cells are able to secrete TNF superfamily member 14 (TNFSF14), which increases FFA uptake in hepatocytes and induces steatosis. Through interactions with hepatocytes, $CD8^+$ T cells and NKT cells cooperatively induce liver damage and steatosis (100). Obesity decreases the population of $CD4^+$ T cells which play a critical role in NAFLD-HCC progression and loss of hepatic $CD4^+$ T cells compromises immunotherapies, such as RNA vaccine (M30) and anti-OX40 antibody-mediated therapy against tumor cell growth in the liver (101, 102).

2.8 Autophagy

Autophagy is a lysosome-dependent catabolic process that contributes to hepatic homeostasis through its role in energy balance and cytoplasmic quality control, removing misfolded proteins, damaged organelles and lipid droplets (103). Autophagy shows beneficial or deleterious effects, depending on the cell type. Autophagy regulates the breakdown of lipid droplets and prevents liver injury in hepatocytes, exerts anti-inflammatory effects in macrophages, while autophagy has pro-fibrogenic properties in HSC (51, 84, 104).

Growing evidence suggests that autophagy is inactive during obesity and NAFLD. Obesity and its associated metabolic stress can interfere with the autophagic process, leading to the promotion of retention of damaged mitochondria, elevated oxidative stress and activation of DNA damage responses, accelerating obesity-related pathology in the liver, adipose and gut (105). This dysregulation of autophagy has been linked to many liver diseases, including HCC (84). In the obese state, the excess of TG and FFAs inhibit the initiation of autophagy through activation of mammalian target of rapamycin (mTOR) and suppression of unc-51 like autophagy activating kinase 1 (ULK1) activity (106). The decreased autophagic function contributes to hepatic oxidative stress, steatosis and other pathophysiology of HCC (107). Autophagy suppresses tumorigenesis by blocking cell damage or facilitating the removal of tumorigenic initiating cells, and thus, impairment of autophagy may be a causal factor in the development of HCC in advanced NASH. In addition, a change in autophagic activity plays a critical role in the development of immune response, insulin sensitivity, diabetes and other metabolic diseases, which promotes HCC development (107, 108). Collectively, all the above suggests autophagy may be a therapeutic target in obesity-associated HCC.

2.9 Hepatokines and metabolism

Accumulating evidence reveals that obesity accelerates the secretion of hepatokines from hepatocytes such as hepassocin (HPS), angiopoietin-like protein 8 (ANGPTL8), Fetuin-A and B and fibroblast growth factor 19 and 21 (FGF19/21) (109, 110). Hepatokines mainly act as liver-derived pro-inflammatory factors, playing an essential role in inducing liver steatosis and NASH to HCC by modulating the lipid metabolism progress, ROS production, inflammatory response and other oncogenic conditions (111, 112). In turn, hepatic steatosis and HCC induce the secretion of ectopic hepatokines and play an alternative role in the pathogenesis of obesity (96). In addition, the associated metabolic changes caused by hepatokines alter the secretion of other organokines that play a regulatory role in the pathogenesis of NASH (113).

Elevation of these hepatokines in plasma has been associated with HCC development or a poor prognosis in NAFLD-related HCC. For instance, HPS overexpression facilitates hepatic lipid accumulation and promotes inflammatory cytokines and lipogenic gene expression (114). A high concentration of Fetuin-A is associated with IR and enhances the release of pro-inflammatory cytokines, inducing a lipotoxic pro-inflammatory response (112). ANGPTL8 is highly expressed in liver and AT, its overexpression is positively correlated with hepatic steatosis, lipogenesis and tumor cell proliferation (114). Hepatokines may be considered biomarkers of ectopic fat accumulation in the liver and markers of the disease progression, some of them may be the target for the prevention and treatment of IR and HCC (113, 115).

2.10 Genetic factors

The obesogenic environment exposes a disease risk associated with genetic variants, including NAFLD, NASH and HCC. Genetic factors may be responsible for the individual susceptibility and clinical course of NAFLD. Multiple studies have emphasized that specific genetic variations predispose to NAFLD susceptibility and NAFLD-related HCC (31). Single nucleotide polymorphisms (SNPs) in genes, including human patatin-like phospholipase domain-containing 3 (*PNPLA3*), transmembrane 6 superfamily

member 2 (*TM6SF2*), glucokinase regulator (*GCKR*), membrane-bound O-acyltransferase domain-containing 7 (*MBOAT7*), hydroxysteroid 17β-dehydrogenase 13 (*HSD17B13*), are associated with NASH development and they are associated with regulation of hepatic fat content, plasma liver enzyme levels and glucose metabolism (116, 117). For example, an SNP rs738409 C/G in *PNPLA3* results in an isoleucine to methionine substitution at residue 148, which is designated *PNPLA3* I148M. The effect of the *PNPLA3* I148M genetic variation is significant, with each allele having approximately a 2-fold increase in the odds of NAFLD and a 3-fold increase in the odds of NASH and HCC (118). An SNP in *TM6SF2* encoding a glutamate to lysine substitution at amino acid position 167 of TM6SF2 protein (E167K) is associated with increased DNL, reduced secretion of apolipoprotein B particles, promoting the development of NASH, advanced hepatic fibrosis and cirrhosis (118, 119). All SNPs in *TM6SF2, GCKR, MBOAT7* and *HSD17B13* are shown to be associated with *PNPLA3* I148M, affecting all stages of NAFLD, suggesting that these genetic variants have additive effects on the progression of NAFLD and NAFLD-related HCC (84). In addition, obesity interacts with *PNPLA3* I148M genetic variation to elevate liver fat content and NAFLD susceptibility, and to increase the risk of liver injury, liver fibrosis and HCC (120–122). *PNPLA3* I148M has a more severe effect on liver injury in people with obesity than in lean individuals. Obesity also amplifies the interaction of *PNPLA3* I148M with alanine transaminase ALT level and cirrhosis. Other studies also report the interactions of obesity with *TM6SF2* E167K and *GCKR* P446L (123, 124). Genetic variation combined with obesity, increased abdominal fat mass and excessive carbohydrates may confer a higher risk of developing HCC (125).

2.11 Epigenetic modification

Epigenetic regulation of gene expression via DNA methylation, histone modification and microRNAs (miRNAs) are all associated with NAFLD development (126). Epigenetic alterations occur when exposed to an obese or nutrient-rich environment (127). Excessive glucose, lipid and insulin-generated metabolites may disrupt the epigenetic balance, thereby altering transcriptional networks involved in redox homeostasis, peroxisome and mitochondria function, inflammation, insulin sensibility and lipid homeostasis, driving NAFLD development and NAFLD-associated HCC tumorigenesis (126, 128).

DNA methylation is the most reported epigenetic modification (129). Accumulating investigations show the key genes responsible for metabolic, lipid homeostasis, insulin signaling, DNA repair, remodeling of liver tissue and fibrosis progression are significantly and differentially methylated (126) (130). Dipeptidyl peptidase 4 (DPP4), an adipokine released by hepatocytes, is known to be upregulated in the liver of patients with obesity and NAFLD, while methylated *DPP4* is negatively correlated with the stages of hepatic steatosis and NASH (131). A previous study showed that hypermethylation of the peroxisome proliferator-activated receptor gamma (*PPARG*) promoter was associated with fibrosis severity in liver biopsies (132).

Histones undergo various modifications such as acetylation, phosphorylation, methylation, ubiquitination, SUMO-ization and ribosylation, of which acetylation has been extensively reported (133). Histone acetylation promoted by histone acetylase (HAT) activates gene transcription, while histone deacetylation catalyzed by histone deacetylase (HDAC) promotes gene silencing. It has been reported that altered expression and function of HAT and HDAC affect hepatic metabolism and cellular transformation in NAFLD (134). P3000, a member of the HAT family, is involved in regulating the transcription of the NF-κB pathway, glycolytic and lipogenic genes, and contributes to hepatic steatosis and NAFLD development (128, 133). One study found that inhibition of p300 improved MAFLD in mice, restored biochemical parameters and reduced the activity of genes involved in adipogenesis (135).

It is well documented that several miRNAs are considered to be critical mediators of metabolic diseases including obesity, T2DM, metabolic syndrome and MAFLD (136, 137). These miRNAs encompass miR-27b, miR-33, miR-34a, miR-103, miR-107, miR-122 and miR-223, which play an essential role in controlling the metabolism and homeostasis of insulin, glucose, cholesterol and lipid (128). miR-122 is a liver-rich and liver-specific miRNA with key roles in liver metabolism, cholesterol biosynthesis, fatty acid synthesis and oxidation (138). Systemic or specific deletion of miR-122 in the liver showed a significant decrease in total serum cholesterol (TC) and TG levels and a marked improvement in hepatic steatosis, suggesting that miR-122 is a crucial regulator of cholesterol and fatty acid metabolism in the liver and a potential therapeutic target for NAFLD (128, 138).

2.12 Fatty metamorphosis

Fatty metamorphosis is a prominent histologic characteristic of well-differentiated HCCs, and HCCs show various degrees of fatty metamorphosis (139, 140). Fatty metamorphosis can be classified as diffuse and focal forms. Diffuse metamorphosis is found throughout the cancer nodule, whereas focal metamorphosis localizes in part of the nodule. The frequency of fatty metamorphosis is highest in HCCs with a diameter of 11-15 mm, and the type of metamorphosis may transition from diffuse to focal (140). Some studies suggest that the possibility of HCC should be considered when focal fatty metamorphosis is found in the cirrhotic liver (139). Fatty metamorphosis is thought to be related to ischemia and metabolic disorder, including obesity, diabetes and hyperlipidemia (141). In hepatic fatty metamorphosis, triglycerides are deposited in the hepatocytes, effectively converting the cells into adipocytes (142). A study found that the severity of fatty metamorphosis is increased from normoglycaemic to diabetic obese patients (143).

3 Molecular mechanisms of obesity-associated HCC

3.1 IGF pathway

The IGF axis consists of three ligands (insulin, IGF-1 and IGF-2), three receptors (insulin receptor, IGF-1R and IGF-2R),

substrates (insulin receptor substrate IRS) as well as ligand binding proteins (144). Dysregulation of IGF signaling plays a critical role in the pathogenesis and carcinogenic of HCC, particularly in obesity-associated HCC (31). Current evidence indicates that insulin and hyperinsulinemia promote the synthesis and biological activity of IGF-1 and IGF-2, which regulates the energy-dependent growth process (31, 36).

IGF-1 is mainly secreted by the liver, and it can act as an autocrine, paracrine or endocrine growth factor. IGF-1 has a higher affinity for IGF-1R, which is involved in the generation of preneoplastic lesions (145). The binding of IGF-1 and IGF-1R is able to regulate stem cell pluripotency and differentiation, triggering cell proliferation, organ development and tissue regeneration (33). In addition, imbalanced IGF-1/IGF-1R signaling stimulates HCC cell proliferation and inhibits apoptosis through activating MAPK pathway and c-JNK pathway. IGF-1 also promotes angiogenesis by increasing the production of VEGF (146). Plasma level of IGF-2 is increased in obese, T2DM patients, and cirrhosis as well as HCC (144). Similar to IGF-1, IGF-2 is also produced in the liver. During hepatocarcinogenesis, IGF-2 has a variety of oncogenic functions via binding to IGF1R, such as inhibiting apoptosis, promoting HCC cell proliferation and migration, and activating angiogenesis (147). IRS-1, the main substrate of IGF-1R activation, is a key component of IGF axis. Studies have demonstrated IRS-1 acts as a dominant oncogene and has a higher level in HCC (148). Hyperinsulinemia and elevated IGFR activates the phosphorylation of IRS-1, resulting in the activation of multiple cytokine pathways, including PI3K/AKT/mTOR and MAPK cascade, which modulate cell cycle and may potentially enhance tumor progression of HCC (35).

3.2 Wnt/β-catenin pathway

Wnt/β-catenin signaling is one of the most important pathways required for cell fate differentiation and overall maintenance of liver metabolism and homeostasis (149). Dysregulation of the Wnt/β-catenin pathway and its various components effects NAFLD progression, starting with early obesity, diabetes, NASH and progressing to fibrosis, cirrhosis and HCC. In turn, evidence suggests that Wnt activity is enhanced in liver cirrhosis, and it is frequently hyperactivated in HCC patients (149, 150). An aberrant activation of Wnt/β-catenin signaling is a hallmark of various hepatic pathologies, it plays a role in almost every aspect of liver biology (151).

β-Catenin, encoded by *CTNNB1*, is the core protein of the Wnt signaling cascade. Central to the pathway is the interaction of Wnt ligand with Frizzled/low-density lipoprotein receptor-related protein (LRP) co-receptor complex, β-catenin accumulates aberrantly in the nucleus, leading to the expression of many transcriptional targets, including gene responsible for proliferation (e.g. *MYC*), anti-apoptosis (e.g. *BIRC5*), epithelial-mesenchymal transition (e.g. *Snail*), invasion (e.g. *MMPs*), angiogenesis (e.g. *VEGF*), inflammation (e.g. *IL-6*)and stemness (e.g. *SOX2*) (152). β-catenin plays a role in cell-cell adhesion, is a component of adhesion junctions and facilitates the assembly of adhesion junctions (151). HSCs express several different Wnt receptors and various components of Wnt signaling like Wnt3a and Wnt5a promote HSC activation (153), and have been shown to be critical in the onset and progression of fibrosis (149). Thus, activation of the Wnt/β-catenin pathway not only regulates tissue development and regeneration but also affects tumorigenicity and enhances metastatic potential in HCC (147, 154). A growing body of evidence links Wnt/β-catenin to adiposity, body fat distribution and metabolic dysfunction in humans. It can regulate hepatic lipid metabolism and AT function by modulating other regulatory cytokines such as sterol regulatory element-binding protein 1 (SREBP-1), fatty acid synthase (FAS) and the peroxisome proliferator-activated receptor (PPAR) family (155, 156). In addition, Wnt/β-catenin plays a pivotal role in modulating cross-talk between different components of tumour microenvironment (TME), including immune cells, stem cells and non-cellular components of the TME in HCC (157). All of the above support that the Wnt/β-catenin pathway is a potential molecular-targeted therapy in HCC.

3.3 JAK/STAT pathway

The Janus protein tyrosine kinase (JAK)/STAT pathway, is a vital downstream mediator for diverse cytokines (IL-6), hormones (leptin) and growth factors (EGF), and is dysregulated in the context of obesity and metabolic disease, including HCC. JAKs and STATs can regulate adipocyte development, such as adipogenesis and transition from preadipocytes to adipocytes, as well as the function of mature adipocytes, and the persistent activated of STAT can lead to deleterious pathological manifestations (158, 159). Accumulating evidence shows that JAK/STAT pathway involves multiple metabolic processes like insulin sensitivity, gluconeogenesis and adiposity (160, 161).

Adipocyte JAK2 and STAT5 deficiency leads to hepatic lipid accumulation, hepatic steatosis, IR and tumorigenesis (160). Hepatic growth hormone (GH) plays an important role in lipid metabolism, systematic glucose metabolism energy supply and cellular regeneration through activating JAK2/STAT5 pathway. Obesity and excess glucose inhibit the secretion of GH, which disrupts GH/JAK2/STAT5 signaling, resulting in excess hepatic lipid accumulation and promoting the process of NAFLD and subsequent HCC (162). STAT3 is closely related to liver injury and plays a pivotal role in the pathogenesis of liver diseases. IL-6 is the most well-described activator of STAT3. The activation of IL-6/JAK/STAT3 signaling in the liver promotes the development of obesity-associated HCC through exacerbating metabolic stress-induced inflammation and immune response (163). Intriguingly, obesity-driving NASH and HCC depend on different STAT signaling pathways (81). In the context of obesity, the oxidative hepatic environment inactivates T cell protein tyrosine phosphatase (TCPTP), a negative regulator of STAT1 and STAT3, and increases STAT1 and STAT3 activity. While the enhanced STAT1 facilitates the recruitment of activated cytotoxic T cells and the consequent NASH and fibrosis. Conversely, STAT3 promotes HCC in obese patients, independent of T cell recruitment, NASH and fibrosis (164). In addition, JAK/STAT signaling controls a diversity of

cellular functions, including cell proliferation, stem cell maintenance, differentiation, invasion and metastasis (165).

3.4 PI3K/AKT pathway

In obesity, T2DM and NAFLD, hyperinsulinemia and dysregulated insulin signaling occurs when insulin and IGF-1 bind to their respective receptors and activate PI3K/Akt signaling, a key oncogenic pathway for metabolism, cell growth and cell survival (66, 88). In turn, the dysregulated PI3K/Akt pathway further exacerbates the development of obesity, T2DM and subsequent HCC.

AKT regulates lipid metabolism and hepatic lipid content homeostasis. The PI3K/AKT signaling pathway stimulates the gene expression of proteins and transcription factors involved in DNL, including acetyl-CoA carboxylase alpha (ACCα) and sterol regulatory element binding transcription factor 1 (SREBP1) (166). Overexpression of AKT increases glucose uptake, and PI3K/AKT-mediated dysfunction of glucose transport and glycogen synthesis plays an important role in the development of obesity and T2DM (167). AKT2 is a major AKT isoform expressed in insulin-sensitive tissues like liver, its liver-specific deletion inhibits hepatic TG accumulation, further supporting the importance of PI3K/AKT signaling activation in obesity-associated HCC (168). Phosphate and tensin homolog (PTEN), a negative regulator of the PI3K/Akt pathway, suppresses the expression of enzymes involved in hepatic DNL and IR. PTEN is downregulated in the livers of NASH and HCC patients, the deletion of PTEN activates PI3K/AKT, and elevates the levels of SREBP-1c and lipogenic genes, promoting the development of NASH and HCC (88).

3.5 MAPK pathway

The family of mitogen-activated protein kinases (MAPKs) mainly includes the stress-responsive MAPKs, c-JNK and p38 MAPK. The associated inflammatory state in obese and insulin-responsive tissues activates stress-responsive p38 MAPKs and JNKs. MAPKs play a prominent role in regulating diversity metabolism processes (169).

JNK is highly activated in NASH-HCC, and the activation of JNK is related to the degree of liver histology activity and promotes the development and carcinogenesis of NASH (69). In obesity and hyperinsulinemia, the increased FFAs, ROS and TNF-α lead to JNK activation in hepatocytes and macrophages, which can increase the production of inflammatory cytokines that can cause inflammation, apoptosis, hepatic IR, liver injury and fibrosis, supporting the metabolic contribution of JNK pathway (66, 170). JNK hyperactivation in macrophages is required for tissue infiltration and pro-inflammatory differentiation, the JNK1 deficiency in macrophages leads to a protective effect against the development of hepatic IR (171). JNK is directly involved in the inhibition of fatty acid oxidation and susceptibility to steatosis through the inhibition of hepatic PPARα and its target genes. In addition, JNK is involved in lipotoxicity and triggers the apoptosis pathway by activating proapoptotic proteins like Bcl-2-like protein 11 (Bcl2-L-11), Bcl2-associated agonist of cell death (BAD) and Bcl-2-like protein 4 (Bcl2-L-4) (40).

Hepatic p38α/β MAPK stimulates hepatic gluconeogenesis by driving the activation of gluconeogenic genes including phosphoenolpyruvate carboxy kinase (PEPCK), glucose-6-phosphatase (G6Pase), and peroxisome proliferator-activated receptor gamma coactivator-1A (PGC-1α) (170). Recent studies demonstrate that activation of p38α MAPK promotes ER, IR and accelerates NASH pathogenesis (169) as well as being elevated in obese patients with NAFLD (172).

3.6 AMPK pathway

AMP-activated protein kinase (AMPK) is an intracellular energy sensor that plays a vital role in maintaining energy homeostasis and is involved in diverse biological processes. AMPK activity is increased by nutrient deprivation and reduced in response to inflammation, obesity and NAFLD (52). Loss of AMPK activity exacerbates liver injury and hepatic fibrosis, while increasing AMPK activity has been viewed as a viable therapeutic strategy to improve NAFLD and decrease the risk of NASH, cirrhosis and HCC via three mechanisms: i) suppression of DNL in liver, ii) increased FFA β-oxidation and iii) promotion of mitochondrial function/integrity in AT (88, 173).

In obese humans, ablation of AMPK activity in AT leads to IR and increased liver lipid accumulation (173). In macrophages, AMPK promotes anti-inflammatory phenotypes by inhibiting NF-κB and JNK-mediated pathways, and alleviates the expression of pro-inflammatory genes, such as CCL2 and TNF-α. Activation of AMPK ameliorates liver fibrosis through a variety of mechanisms, including reducing the stimulation of fibrosis, preventing HSCs activation/proliferation/migration and inhibiting the expression of fibrotic genes (174). In addition, AMPK regulates cell proliferation through inhibiting mTOR signaling. Accumulating evidence confirms that hepatic AMPK activity is greatly diminished in NAFLD and NASH, while liver-specific activation of AMPK reduces adipogenesis and completely protects against hepatic steatosis and fibrosis *in vivo* (175).

3.7 NF-κB and toll-like receptor pathways

NF-κB and toll-like receptors (TLRs) are key inflammatory pathways associated with obesity-associated HCC (176). Obesity-associated chronic low-grade inflammation is partly mediated by saturated fatty acids stimulating pro-inflammatory pathways in a TLR4-dependent manner in adipocytes and macrophages (40). TLR signaling is able to activate transcription factors (NF-κB and AP-1) and promotes the secretion of inflammatory cytokines (IL-6, IL-1β and TNF-α). These high levels of pro-inflammatory cytokines in hepatocytes cause IR, hepatocytes injury and promote NAFLD, NASH and HCC progression. The elevated IL-1β in KCs, regulated by TLR4, leads to steatosis, inflammation and fibrosis (177). The effect of TLRs on the gut microbiota is an important factor in the

relationship between inflammation and obesity. One study shows that mice with TLR5 deficiency have a unique gut microbiota that makes them sensitive to obesity and metabolic syndrome (178).

NF-κB is a transcription factor that plays crucial roles in inflammation, immunity, cell proliferation and the development of liver injury, fibrosis and HCC (179). IKKα/IKKβ complex directly activates NF-κB and is associated with the gene expression downstream of TLRs and cytokines. In the context of obesity, the activation of NF-κB in hepatocytes contributed to IR, increased FFAs, and glucose intolerance (178). In turn, FFA flux can activate NF-κB, via promoting hepatic lipotoxicity, suggesting a potential link between elevated circulating or tissue lipid concentrations and the part of the immune system that mediates inflammation (66). NF-κB has a wide range of functions in different cellular compartments, affecting hepatocyte survival, inflammation in KCs, and survival, inflammation and activation of HSCs (180). For instance, NF-κB participates in activating HSCs and promotes pro-fibrogenic HSC phenotype. NF-κB plays a pivotal role in modulating HSCs survival and promoting the induction and secretion of inflammatory chemokines, including CCL2 and CCL3 (181). On the other hand, NF-KB plays a protective role in the liver and the pronounced inhibition of NF-KB leads to apoptosis of hepatocytes (180). Genetic models lacking major regulators of NF-κB activation such as *Ikkβ*$^{-/-}$ and *Nemo*$^{-/-}$ resulted in a severe embryonic lethality phenotype with significant hepatocyte apoptosis (182).

3.8 p53 pathway

The tumor suppressor gene *p53* has emerged as an important regulator of hepatic homeostasis and dysfunction through the integration of cellular stress responses, metabolism and cell cycle regulation, which plays a vital role in the pathogenesis of NAFLD and NASH (154). Under normal circumstances, moderate and temporary p53 activation inhibits the accumulation of liver lipids and inflammation. While exposed to sources of cellular stress such as NASH and overnutrition, the hyper-activation of p53 triggers IR, lipid accumulation, inflammation and oxidative stress in different ways, increasing the risk of HCC (183, 184).

In the context of obesity and hyperglycemia, p53 expression is increased. Elevated p53 level exacerbates the release of pro-inflammatory cytokines and leads to metabolic abnormalities that contribute to the development and progression of HCC (185, 186). For example, in the presence of hyperglycaemia or excessive calorie intake, p53 is activated and leads to systemic IR (187). High p53 levels, whether induced as a response to adiposity or as a trigger for adiposity, may be counterproductive to maintaining AT homeostasis. Recent studies highlight that p53 is essential for regulating the formation of white and brown AT and is also a suppressor of pre-differentiation of adipocytes (187). In AT, the activation of p53 promotes the expression of pro-inflammatory adipokines through NF-κB signaling, leading to hepatic steatosis, IR and diabetes, while inhibition of p53 activity impairs inflammation and attenuates hepatic steatosis (185, 188). p53 is also a major positive regulator of hepatocyte lipid metabolism, and it is involved in lipotoxicity-mediated NASH progression (189). In addition, activation of p53 increases apoptosis of hepatocytes, leading to HSCs activation and the development of liver fibrosis, whereas ablation of p53 completely abolishes this fibrotic phenotype (190).

4 Preclinical animal models of NAFLD, NASH and HCC

Human data on liver disease progression is sparse and often limited to a single point in time due to limited access to liver tissue. *In vitro* models do not fully reflect the hepatic and extrahepatic conditions of human NASH. To better understand the pathogenic mechanisms and develop innovative therapies for human obesity-associated HCC, preclinical experimental animal models have been developed to mimic the major features of NAFLD/NASH/HCC, including genetic, metabolic, histologic, as well as proteomic, lipidomic and transcriptomic changes (84). To date, animal models of NAFLD/NASH/HCC can be roughly classified as diet-induced, genetic, toxic or a combination of more than one intervention (Table 1).

4.1 Dietary animal models

A diet-induced obesity model, whose macro-nutritional profile is similar to that of obese humans, is the popular NASH mouse model (207). Methionine and choline-deficient (MCD) diet is the most frequently used diet to induce measurable hallmarks of NAFLD and produce the most severe phenotype of NASH in the shortest time. This diet is high in sucrose (40%) and fat (10%), and is deficient in methionine and choline, which are crucial for hepatic β-oxidation and the release of VLDL. In addition, MCD alters glucose metabolism, increases fat accumulation in the liver, and induces significant fibrosis and liver injury (212). However, the MCD model is associated with weight loss, lacking systemic IR, no residual AT and no HCC occurrence (191).

An alternative model uses the Choline-deficient L-amino-defined (CDAA) diet, which is deficient in choline, but proteins in the formula are replaced with an equivalent and corresponding mixture of L-amino acids. Similar to MCD diet, CDAA promotes lipid synthesis, inflammation, steatohepatitis, liver fibrosis and HCC (200). After prolonged CDAA feeding, mice develop obesity, IR and elevated plasma TG and cholesterol (213).

A high-fat diet (HFD) composed of 71% fat, 11% carbohydrates and 18% proteins, can directly increase hepatic FFA accumulation and trigger mitochondrial dysfunction. The HFD model is known to develop IR, inflammation, hepatocyte apoptosis, NASH and fibrosis (194).

Western diet (WD) contains 21.1% fat, 41% sucrose, and 1.25% cholesterol, supplemented with high sugar solution (23.1 g/L d-fructose plus 18.9 g/L d-glucose) in drinking water (202). WD induces obesity, IR and dyslipidemia, activates inflammatory, apoptotic and fibrogenic pathways, and progresses NAFLD, fibrosis, NASH and HCC (212).

TABLE 1 Animal models for NAFLD, NASH and HCC.

Model	obesity	IR	NAFLD	NASH	Fibrosis	HCC	Refs
Diet models							
MCD	No	No	Yes	Yes	Yes	No	(191, 192)
CDAA	No	Yes	Yes	Yes	Yes	Low probability	(193)
HFD	Yes	Yes	Yes	Yes	Yes	No	(194)
WD	Yes	Yes	Yes	Yes	Yes	Yes (8-13 months)	(195)
HFD + MCD						Yes	(196)
HFD + CDAA	Yes	Yes	Yes	Yes	Yes	Yes (24-36 weeks)	(84)
HFD + fructose	Yes	Yes	Yes	Yes	Yes	No	(197)
ALIOS	Yes	Yes	Yes	Yes	Yes	Yes (more than 12 months)	(196, 198, 199)
Diet & Toxin models							
STAM	No	No	Yes	Yes	Yes	Yes (16 weeks)	(194, 200)
HFD + DEN	Yes	Yes	Yes	Yes	Yes	Yes (9 months)	(201)
CDAA-HFD + DEN	Yes	Yes	Yes	No	No	Yes (20 weeks)	(84)
WD + CCl4	Yes	Yes	Yes	Yes	Yes	Yes (24 weeks)	(202)
Genetic & Diet models							
ob/ob mice + MCD or HFD	Yes	Yes	Yes	Yes	No	No	(199, 203)
db/db mice + MCD	Yes	Yes	Yes	Yes	Yes	No	(199, 203)
foz/foz mice + WD	Yes	Yes	Yes	Yes	Yes	Yes (56 weeks)	(84, 204)
Pparα$^{-/-}$ mice + HFD	Yes	No	Yes	Yes	Yes	Yes (24 weeks)	(84)
Mc4r$^{-/-}$ mice +HFD	Yes	Yes	Yes	Yes	Yes	Yes (48 weeks)	(84, 205)
Hnf4α$^{-/-}$ mice + HFD	Yes	No	Yes	Yes	Yes	Yes (36 weeks)	(84, 206)
MUP-uPA transgenic + HFD	Yes	Yes	Yes	Yes	Yes	Yes (40 weeks)	(207, 208)
Genetic models							
Pten $^{-/-}$ mice (hepatocyte specific)	No	Yes	Yes	Yes	Yes	Yes (40 weeks)	(84, 196)
Alr$^{-/-}$ mice	No	No	Yes	Yes	No	Yes (12 months)	(209, 210)
Aox $^{-/-}$ mice	No	No	Yes	Yes	Yes	Yes (60 weeks)	(84)
Mat1a$^{-/-}$ mice	No	No	Yes	Yes	Yes	Yes (72 weeks)	(84)
Srebp-1c transgenic	No	Yes	Yes	Yes	Yes	Yes	(207, 211)

Finally, the American lifestyle induced obesity syndrome (ALIOS) diet model is enriched in trans-fats (30% of fat content) and fructose (applied by corn syrup-containing drinking water) (213). In ALIOS model, the hepatic expression of lipid metabolism and insulin signaling genes are increased. In addition, ALIOS induces liver inflammation and bridging fibrosis. Mice in ALIOS exhibit early NASH at 6 months and hepatocellular neoplasms after 12 months (198).

4.2 Diet plus toxin animal models

Streptozotocin (STZ) is a naturally occurring chemical that is toxic to insulin-secreting β cells and is often used in preclinical settings to induce type I diabetes (209). It may also directly cause insulin-independent hepatotoxic effects. The combination of STZ with HFD generates a STAM model. In this model, mice are given a low-dose of STZ through intraperitoneal or subcutaneous injection shortly after birth and then feed HFD at 4 weeks of age. This model leads to simple steatosis after 5 weeks, NASH after 7 weeks, followed by fibrosis after 9 weeks, adenomas after 12 weeks and evidence of HCC at approximately 16 weeks (196). The STAM model therefore rapidly induces NASH, however, the mice are lean and have insulin deficiency. Its pathological mechanisms are different from human NASH (200).

Diethylnitrosamine (DEN) is also a chemical to model HCC in mice. DEN induces severe oxidative stress and DNA damage, and promotes lipotoxicity and liver fibrosis. The combination of DEN

with HFD or with HFD+CD leads to the occurrence of NASH-associated HCC. In this model, the treated mice exhibit obesity and hepatic steatosis after 8 weeks, IR, lobular inflammation and fibrosis after 12 weeks, and develop into HCC within 20 weeks (200). DEN is a procarcinogen and may be relevant in carcinogenesis, this may represent a substantial difference from its human counterpart (196).

As a hepatotoxin, carbon tetrachloride (CCl4) has been broadly used for inducing liver injury and fibrosis in mice (202). CCl4 triggers oxidative stress and necrotic responses in the liver, leading to liver injury, inflammation and excessive activation and proliferation of HSCs (200). The combination of WD and weekly CCl4 has the advantage of rapid disease progression as mice develop stage III fibrosis at 12 weeks and HCC at 24 weeks (209). More importantly, transcriptome analysis revealed close similarities between the model and human NASH. The CCl4 model can be used to study the progression from simple steatosis to NASH to cirrhosis and HCC (200).

4.3 Diet plus genetic animal models

Leptin deficiency (*ob/ob* mice) has been a frequently used model of general metabolism and NAFLD research for a long time (199). *Ob/ob* mice are hyperphagic, inactive and develop severe obesity, hyperlipidemia, hyperglycemia, hyperinsulinemia, and IR, but do not progress to severe liver damage and NASH on a normal diet unless fed with HFD or MCD (214). However, *ob/ob* mice are resistant to liver fibrosis due to leptin requirement (215). Leptin receptor deficiency (*db/db* mice) carries a spontaneous mutation in the *db* gene encoding the leptin receptor, which leads to defective leptin signaling (203). Similar to *ob/ob* mice, *db/db* mice are hyperphagic, obese and IR, and spontaneously develop liver steatosis under normal dietary conditions. *Db/db* mice alone are good models for NAFLD, but not for NASH. However, NASH can be induced when the *db/db* mice are fed with MCD diet. Unlike *ob/ob* mice, *db/db* mice are more susceptible to liver fibrosis (214). In addition, spontaneous mutations in the Alstrom syndrome 1 gene encoding for a protein localized to centrosomes and appetite-sensing neuronal cilia (*foz/foz* mice), *Ppar*$\alpha^{-/-}$ knockout mice, and melanocortin receptor 4 knockout (*Mc4r*$^{-/-}$ mice) lead to overeating, obesity and IR, but do not progress NASH or HCC. HCC is induced in *Mc4r*$^{-/-}$ mice and liver-specific *Hnf4*α-deficient mice feeding with HFD within 1 year and 36 weeks, respectively (205, 206). The *foz/foz* mice fed with a WD will present HCC features for more than 56 weeks (204).

Other genetic models like the *Pten* null mice, acyl-coenzyme A oxidase (*Aox*), and methionine adenosyltransferase 1A (*Mat1a*) in global-deficient mice present HCC under normal diet, but show limitations such as no obese phenotype (84).

While the findings from these animal models facilitate our understanding of the pathophysiology of NASH and NAFLD-associated HCC, systematic transcriptome profiling of liver tissues has revealed changes induced by some dietary or genetic models that are not fully mimic the transcriptional profiling of human NASH (106, 216). Due to the complex pathophysiology involved in NAFLD, the ideal animal model representing the complete NAFLD spectrum within a feasible time frame does not exist (217). Researchers should choose the most suitable animal model according to their research objectives, taking into account the comorbidities of NAFLD, the grade of fibrosis and the possible development of HCC.

TABLE 2 Serum markers and score systems for diagnosing NAFLD, NASH and early-stage HCC.

Marker/Score	Parameters	Diagnosis	Refs
Markers			
TG/HDL-C ratio	TG, HDL-C	NAFLD	(227)
BGN	The cutoff value of 189.58 pg/mL of serum BGN with the best sensitivity (93.55%) and specificity (87.18%)	Fibrosis stage of NASH	(228)
CK18	CK18	NAFLD and fibrosis	(229)
Pro-C3	Pro-C3	NASH and different fibrosis stages	(230)
PIIINP	PIIINP	NASH and different fibrosis stages	(34)
Inter-Alpha-Trypsin Inhibitor Heavy Chain 4 (ITIH4)	ITIH4	HCC with NAFLD	(81)
AFP	Ultrasound, AFP	Early stage-HCC with cirrhosis	(221)
Score system			
SteatoTest™	Alanine transaminase (ALT), a2-macroglobulin (A2M), apolipoprotein A-1 (ApoA1), haptoglobin, total bilirubin, gamma-glutamyl transferase (GGT), TC, TG, age gender and BMI	Different stages of steatosis in NAFLD	(218, 231)
NAFLD ridge score	ALT, HDL-C, TG, haemoglobin A1c, white blood cell count, hypertension	NAFLD	(232)
Fatty liver index (FLI)	BMI, TG, waist circumference (WC), and GGT	NAFLD	(233)

(Continued)

TABLE 2 Continued

Marker/Score	Parameters	Diagnosis	Refs
Hepatic steatosis index (HSI)	8× (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes mellitus)	NAFLD	(154, 234)
BARD score	BMI, AST/ALT ratio, diabetes, diabetes mellius	NAFLD	(154)
APRI	AST, platelets	NAFLD	(235)
AUROC	Waist circumference, ALT, HbA1c, and HOMA-IR	NAFLD	(154)
Lipid accumulation product (LAP)	WC, TG and gender	Different stages of fibrosis in NAFLD	(225, 236)
NAFLD liver fat score (NAFLD-LFS)	Diabetes, insulin, AST/ALT	Different stages of Fibrosis in NAFLD	(225, 237)
NAFLD fibrosis score (NFS)	Age, hyperglycemia, BMI, platelet count, albumin, AST/ALT ration	Presence of fibrosis in NAFLD	(225)
Index of NASH (ION)	Male: waist-to-hip ratio, TG, ALT and HOMA Female: TG, ALT and HOMA	NASH	(238)
Enhanced Liver Fibrosis (ELF) score	Hyaluronic acid (HA), Tissue inhibitor metalloproteinase 1(TIMP1), and Aminoterminal peptide of procollagen 3 (PIIINP)	Presence of advanced fibrosis in NAFLD	(222)
NASH NIS4	Alpha2 macrogobulin (A2M), Haemooglobin A1c (HbA1c), and Chitinase-3-like protein 1 (CHI3L1)	NASH and advanced fibrosis	(222)
GALAD score	Gender, age, AFP, AFP-L3 and Des-gamma-carboxy-prothrombin (DCP)	Early stage-HCC with NASH	(239)

5 Blood-based biomarkers

A key challenge in managing patients with NAFLD is to differentiate NASH from isolated steatosis, as the former carries a high risk of developing cirrhosis and its complications, such as liver failure and HCC. Liver biopsy is the current gold standard for diagnosing NAFLD and NASH, although it is impractical and invasive, may cause life-threatening complications and result in improper diagnosis due to sampling variability (154, 218). Ultrasound has been the main HCC surveillance test for nearly two decades. However, recent data have shown that ultrasound surveillance alone has limitations, including low sensitivity in detecting HCC at an early stage (219). Combining ultrasound with biomarkers, such as alpha-fetoprotein (AFP) may improve the accuracy of early HCC detection (220, 221). Thus, more biomarkers are needed to diagnose NAFLD, NASH with advanced fibrosis and early-stage HCC, which is critically essential for selecting appropriate treatment (81, 222).

During the last decades, diverse non-invasive blood testing has been developed, plasma biomarkers (e.g. high-density lipoprotein cholesterol (HDL-C), biglycan (BGN), cytokeratin 18 (CK18), pro collagen III (Pro-C3) and plasma N-terminal propeptide of type III procollagen (PIIINP)) are commonly used to reflect specific and complex molecular mechanisms underlying the pathogenesis and progression of NAFLD and NASH (154, 223). Currently, AFP is the only biomarker that has completed all phases of biomarker evaluation and has sufficient evidence to be used in clinical HCC detection when combined with ultrasound (224). Serum inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4) is another potential biomarker for NAFLD progression and HCC development (81). Because the pathogenesis of NAFLD and NASH is complex and may involve multiple biological aberrations, it is unlikely that a single biomarker can differentiate simple steatosis from NASH, and identify advanced fibrosis (225). Therefore, composite score systems (e.g. SteatoTest™, Fatty liver index (FLI), BARD score) include at least two or more variables to increase the robustness of the non-invasive predictive model (223). They are certainly clinically useful and avoid liver biopsy in many cases (226). It is worth noting that the GALAD score based on gender, age, AFP, AFP isoform L3 (AFP-L3), and des-gamma-carboxy prothrombin (DCP) has been approved to be used for the early diagnosis of HCC with NASH (81). Here, the serum markers and score systems for NAFLD, NASH and HCC are summarized in Table 2.

6 Potential therapeutic targets of obesity-associated HCC

6.1 Leptin

Leptin, a predominant adipokine secreted mainly by AT, is increased in obese populations and patients with liver disease and is related to NAFLD progression (240). Leptin is central to the obesity-cancer link since it is produced in proportion to fat mass. Leptin is effective in inducing HCC cells mitosis, growth and motility by activating JAK/STAT, PI3K/Akt and ERK signaling pathways. These pathways upregulate cyclin D1 expression, promoting the proliferation of hepatocytes and HCC cells (241–243). Leptin has a pro-inflammatory effect and a high level of leptin causes other inflammatory cells to stimulate the differentiation of monocytes into macrophages, favoring the chronic inflammatory state associated with obesity (244). Leptin also contributes to hepatic fibrogenesis via TGF-β and activating HSCs (245). In addition, high levels of leptin promote angiogenesis through

upregulating VEGF. In obese individuals, higher levels of leptin increase the risk of HCC recurrence after curative therapy (246).

6.2 Adiponectin

Adiponectin is the most abundant and adipose-specific adipokine secreted by adipocytes, whose reduction plays a central role in obesity-associated HCC. Its level paradoxically increased with the decreasing fat mass (247), and both serum and hepatic levels of adiponectin are decreased in NASH patients (248, 249). It exhibits an anti-inflammatory effect through inhibiting the secretion and action of TNF-α, IL-6 and other proinflammatory cytokines, blocking the activation of NF-κB (250). Adiponectin also displays anti-lipotoxic effects, it is able to promote FFA β-oxidation, prevent lipid accumulation in adipose and hepatic tissues, and regulate glucose homeostasis and hepatic insulin sensitivity (251, 252). Adiponectin exerts an inhibition in the proliferation of adipocyte cells, endothelial cells and tumor cells by activating AMPK and regulating c-JNK/caspase 3 pathways (46). In addition, adiponectin also possesses antiangiogenic properties by decreasing the expression of VEGF. A low level of adiponectin is related to obesity-associated IR and carcinogenesis (253). Hence, it is a novel therapeutic target for obesity-associated HCC (254).

6.3 Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) regulate lipid and glucose metabolism and play a key role in hepatic energy homeostasis and the regulation of adipogenesis (52, 154). PPARα negatively regulates hepatic lipid uptake by regulating FFAs transport, esterifying FFA and increasing mitochondrial FFA oxidation. Activation of PPARα inhibits NF-κB-induced inflammatory genes and reduces the level of acute inflammation response genes (255). Therefore, its abnormalities may cause hepatic steatosis, steatohepatitis, fibrosis, and HCC (52). In addition, PPARα enhances the expression of FGF21 and glutamate transporter 1 (GLT1), which improve systemic insulin sensitivity and lipid turnover (256). Activation of PPARβ/δ protects against dyslipidemia, IR, obesity and NAFLD. PPARβ/δ promotes hepatic glucose catabolism and increases HDL cholesterol and shows a strong TAG- decreasing effect. Similar to PPARα, PPARβ/δ has anti-inflammatory effects in the liver by inhibiting NF-κB activity (256). PPARγ is highly expressed in AT and macrophages and plays a key role in adipogenesis, lipid metabolism, insulin sensitivity and immune regulation (257). PPARγ prevents the increased flow of FFAs and adipokines from AT to other organs, especially to the liver (258). The PPARγ activator, rosiglitazone approved by the FDA for the treatment of T2DM, showed effects against steatosis, hepatocellular inflammation, ballooning degeneration and fibrosis (154).

Elafibranor, a PPARα and PPARβ/δ agonist, improves serum lipid profile and IR and improves NASH without worsening fibrosis in Phase II clinical trials (259). Saroglitazar, a major PPARα and moderate PPARγ agonist, has also been reported to improve liver enzymes, liver fat content, IR and atherosclerotic dyslipidaemia in participants with NAFLD/NASH (260). Lanifibranor, a pan-PPAR agonist, ameliorates diet-induced NASH through upregulation of β-oxidation and FA desaturation (47).

6.4 Farnesoid X receptor

Farnesoid X receptor (FXR), a bile acids-activated nuclear receptor, is highly expressed in intestine, liver and kidneys. FXR is responsible for hepatic glucose and lipid metabolism, carbohydrate metabolism, inflammation, bile acid production, as well as lipoprotein composition, immune responses and insulin signaling (106, 114). More importantly, the excessive activation of FXR triggers a steady release of FGF19, which is an atypical hormonal regulator of metabolism and bile acid homeostasis that has been associated with improvements in NASH (38). In preclinical studies, FXR activation has been shown to attenuate hepatic steatosis, reduce lipotoxicity and inflammation, increase insulin sensitivity, and exhibit direct anti-inflammatory and antifibrotic effects, suggesting that modulation of FXR has beneficial effects on obesity-related liver diseases (207).

Obeticholic acid (OCA) is one of the FXR agonists that has reached Phase III clinical trial. OCA exhibits excellent effects in NASH patients, it improves hepatic inflammation, fibrosis and hepatic damage (261). Cilofexor (GS-9674), a non-steroidal agonist of FXR with anti-inflammatory and anti-fibrotic effects, has completed a Phase II clinical trial. Cilofexor significantly improved hepatic steatosis and reduced serum γ-glutamyl transferase, C4 and primary bile acids in NASH patients, but did not improve liver fibrosis and stiffness (154). Other FXR agonists include tropifexor (LJN452), TERN-101, EDP-305, EYP001a and LMB763 (47, 52).

6.5 Stearoyl-CoA desaturase 1

Stearoyl-CoA desaturase 1 (SCD1), a key enzyme in DNL and fatty acid metabolism, controls a rate-limiting step in mono-unsaturated fatty acid synthesis and has been considered a promising target for NAFLD treatment (262). Obesity and hepatic steatosis are known to strongly induce SCD1 expression, whereas rodents that are specifically deficient in SCD1 in the liver are protected from developing hepatic steatosis by reducing lipid synthesis and increasing FFA β-oxidation and insulin sensitivity (38). Inhibition of SCD1 produces a number of beneficial effects, including reducing liver fat, preventing IR and obesity. Aramchol, an oral SCD1 inhibitor targeting liver, decreased the liver fat content and improved liver histology in a Phase II clinical trial without exhibiting toxicity. Aramchol is being further evaluated as a drug candidate for the treatment of NAFLD in an ongoing Phase III trial (114). In addition, a number of synthetic SCD1 inhibitors, including CVT-12012, GSK1940029, MF-438, MK-8245 and SW203668, are being evaluated for efficacy in preclinical and clinical studies (52).

6.6 Acetyl-CoA carboxylase

It is known that increased hepatic DNL contributes to NASH, while the rate-limiting step in DNL is catalyzed by Acetyl-CoA

carboxylase (ACC), suggesting inhibition of ACC represents an attractive approach for the treatment of NASH (263). ACC has two major isoforms, ACC1 and ACC2. ACC1 is localized on the cell membrane and is expressed in liver and AT, whereas ACC2 is expressed on the mitochondrial surface of oxidative tissues, such as liver, heart and skeletal muscle (47). Inhibition of ACC1 and ACC2 reduces DNL and increases FA β-oxidation. Firsocostat (GS-0976) is a hepatic ACC1 and ACC2 inhibitor that reduces steatosis, inhibits DNL and reduces serum fibrosis markers in non-cirrhotic NASH patients in a Phase II trial (264).

6.7 Fatty acid synthase

Fatty acid synthase (FASN) catalyzes the conversion of malonyl CoA and acetyl CoA to the saturated C16 fatty acid palmitate, which plays a key role in DNL, making this multi-catalytic protein an attractive therapeutic target for obesity, and associated liver diseases (265). FASN inhibition decreases TG content, consistent with direct anti-steatotic activity. Denifanstat (TVB-2640), an inhibitor of FASN, is in a Phase II clinical trial for NASH and is being used in the primary human liver microtissue (LMT) study (266).

6.8 Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) is an endogenous hormone, secreted by intestinal endocrine L-cells that regulates blood glucose levels. GLP-1 enhances the release of insulin, induces fatty acid oxidation in hepatocytes, inhibits glucagon secretion and reduces food intake by binding to the GLP-1 receptor (GLP-1R). Inactivation of GLP-1 leads to glucose intolerance, T2DM and hepatic steatosis (52), suggesting GLP-1 is a potential medication for NAFLD. GLP-1 activity is significantly decreased due to the actions of a protease DPP4, which cleaves GLP-1 and has a higher level in NASH patients (38, 52). Exenatide, the first GLP-1 analogue, is resistant to DPP4 and its secondary and tertiary structures, with a much longer half-life and hypoglycemic effect (52). It is able to decrease serum ALT levels, and improve hepatic fat and fibrosis (267). Liraglutide, another GLP-1 agonist requiring daily injection, results in increased insulin sensitivity, decreased DNL, reduced BMI and suppression of lipolysis in patients with NASH (268). Liraglutide is safe, well tolerated and leads to histological resolution of NASH (269). In addition, the DPP4 inhibitors, sitagliptin and evogliptin can prolong the half-life of GLP-1 and improve NASH (52).

6.9 Sodium-Glucose Cotransporter-2

Sodium-Glucose Cotransporter-2 (SGLT2) is expressed almost exclusively in the kidney, where more than 90% of the glucose filtered by the glomerulus is reabsorbed. SGLT-2 is profoundly involved in the regulation of inflammatory responses, fibrogenesis and many intracellular signaling pathways (270). SGLT-2 inhibitors increase glucagon levels, reduce renal reabsorption of glucose, and promote the loss of calories in the urine, with subsequent weight loss (268). Many studies have indicated that SGLT-2 inhibitors improve liver function and liver fibrosis, suggesting SGLT-2 inhibitors hold promise for treating NASH.

In patients with T2DM and NAFLD, inhibition of SGLT2 by dapagliflozin attenuates liver fibrosis and steatosis, and decreases the serum level of DDP4. The safety and efficacy of dapagliflozin in NASH patients is being assessed in a Phase III clinical trial (271). Other SGLT2 inhibitors currently in use, include canagliflozin, ipragliflozin, ertugliflozin and empagliflozin, which have multiple functions in the treatment of NAFLD and T2DM by preventing DNL, hepatic inflammation and apoptosis, and increasing fatty acid oxidation (154).

6.10 Thyroid Hormone Receptor-β

Thyroid Hormone (TH) is involved in myriad essential cellular and organismal functions like hepatic TG and cholesterol metabolism by binding to two Thyroid Hormone Receptor (THR), THR-α and THR-β (38). THR-β is highly expressed in hepatocytes and is responsible for regulating metabolic pathways in the liver that are often compromised in NAFLD. The THR-β level in liver is reduced in patients with NASH (272). Selective engagement of the THR-β subtype in the liver has emerged as a potential approach for the treatment of NASH. Activation of THR-β is able to decrease TG and cholesterol levels, improve insulin sensitivity, reduce apoptosis, increase fat oxidation and promote liver regeneration (262). Resmetirom is a selective THR-β agonist that likely reduces liver fat, enhances fatty acid catabolism and alleviates hepatic steatosis and dyslipidemia. Currently, resmetirom is being evaluated for safety and efficacy in patients with NASH and fibrosis in a Phase III clinical trial (261).

6.11 Fibroblast growth factors 19 and 21

Circulating FGF21 is derived from the liver and is also expressed in several other tissues, such as the pancreas, muscle and adipose (258). FGF21 has been shown to play a vital role in regulating organ metabolism and systematic energy homeostasis, especially hepatic lipid metabolism. FGF21 enhances lipid oxidation, inhibits lipolysis in AT, suppresses DNL in the liver, and improves insulin sensitivity by inhibiting mTOR (114, 273). Deficiency of FGF21 favors the development of steatosis, inflammation, hepatocyte injury and fibrosis in the liver, while administration of FGF21 analogues improves NASH by attenuating these processes (274). Pegbelfermin (BMS-986036), a recombinant FGF21 analogue, has been used in clinical trials for patients with NASH and stage 3 fibrosis. Subcutaneous treatment of pegbelfermin reduces liver fat, and improves biomarkers of metabolic function and fibrosis (261). Efruxifermin, an FC-FGF21 fusion protein, is able to improve NAFLD activity score (NAS) and fibrosis, and reduce body weight and liver fat content in clinical Phase II trials (261).

FGF19 is a gastrointestinal hormone that regulates bile acid synthesis, glucose metabolism and hepatic fatty acid oxidation and is a known downstream regulator of FXR (258). Circulating FGF19

concentration is decreased in NASH patients, but FGF19 can also stimulate tumour progression through activating STAT3 pathway (275). NGM282, a humanized FGF19 analogue, acts in the same way as FXR agonists (47). In clinical trials, NGM282 is able to reduce AST and ALT levels, and improve liver fat content, fibrosis and liver transaminases (47, 52, 258).

6.12 The C-C chemokine receptors 2 and 5

The C-C chemokine receptors 2 and 5 (CCR2 and CCR5) and their respective ligands (CCL2 and CLL3-5) are implicated in the pathogenesis of liver inflammation, immune cell infiltration and fibrosis, leading to the development of NAFLD and NASH (114). Cenicriviroc is a novel dual CCR2 and CCR5 antagonist currently in clinical development for the treatment of liver fibrosis in NASH patients. It blocks overactive inflammatory signals and disrupts signals that activate stellate cells, thus targeting the onset of inflammation and fibrosis (264). In Phase II clinical trial, cenicriviroc exhibited a significant improvement in fibrosis of NASH patients. However, based on the results of the Phase III AURORA trial, it was terminated early due to lack of efficacy (258).

6.13 Galectin-3

Galectin-3 is a β-galactoside binding protein mainly secreted by macrophages. Its expression is increased in NASH, and it is associated with the severity of fibrosis and inflammatory responses (276). Galectin-3 also modulates diverse physiologic and pathologic processes, including cell apoptosis, adhesion, migration and angiogenesis (277). The ablation of Galectin-3 decreases hepatic advanced lipoxidation endproduct (ALE) accumulation and improves inflammation, hepatocyte injury and fibrosis (278). GR-MD-02 is an inhibitor of Galectin-3, which has shown promising results for NASH patients with fibrosis in clinical trials (52).

6.14 Apoptosis signal-regulated kinase 1

Apoptosis signal-regulated kinase 1 (ASK1)1 plays a pivotal role in regulating hepatocyte injury, inflammation, apoptosis and fibrosis in NASH through c-JNK signaling (279). Selonsertib is a first-in-class inhibitor of ASK1 that has been shown to prevent inflammation, fibrosis, excessive apoptosis and progression to cirrhosis in a Phase II clinical trial in patients with NASH and stage 2-3 fibrosis (114). However, the Phase III clinical trial was terminated since it failed to reach primary and secondary endpoints (258).

7 Clinical trials

Currently, there are no drugs that have been approved for NAFLD/NASH treatment, and treating this disease remains a major unmet clinical need (280). However, within the past decade, a number of studies have been investigating new drugs for NASH, improving developments in this area. Consequently, many drugs are now undergoing various stages of clinical trials in NAFLD/NASH patients. Based on the pathophysiologic classification of NASH, these drugs include insulin sensitizers, anti-DNL drugs, lipid-lowering drugs and anti-fibrosis drugs. In addition, other clinical trials for anti-inflammation and anti-apoptosis agents are also ongoing. Below are the current pharmaco-therapeutic options that are in clinical trials (Table 3).

TABLE 3 Ongoing clinical trials for NAFLD/NASH patients.

Medication	Primary mechanism	Inclusion criteria	Clinical trial number	Trial phase
Insulin sensitizer				
Metformin	Insulin sensitizer	NAFLD	NCT01084486	Phase II
Pioglitazone	PPARγ agonist	NASH, NAFLD, T2DM	NCT00994682	Phase IV (completed)
MSDC-0602K	Mitochondrial pyruvate carrier (MPC) inhibitor	NAFLD, NASH	NCT02784444	Phase II
Inhibition of DNL and lipotoxicity				
Aramchol	SCD1 inhibitor	NASH, NAFLD	NCT04104321	Phase III
Firsocostat (GS-0976)	ACC inhibitor	NASH	NCT02856555	Phase II
TVB-2640	FASN inhibitor	NAFLD	NCT04906421	Phase II
Elafibranor	PPARα/β agonist	NASH with fibrosis	NCT02704403	Phase III (terminated due to failure of the predefined primary surrogate efficacy endpoint)
Seladelpar (MBX-8025)	PPARδ agonist	NASH	NCT03551522	Phase II (terminated due to unexpected histological findings)

(Continued)

TABLE 3 Continued

Medication	Primary mechanism	Inclusion criteria	Clinical trial number	Trial phase
Saroglitazar	PPARα/γ agonist	NAFLD	NCT03617263	Phase II
Lanifibranor (IVA337)	Pan PPAR agonist	NASH	NCT03008070	Phase II
Obeticholic Acid (OCA)	FXR agonist	NASH	NCT02548351	Phase III
Cilofexor (GS-9674)	FXR agonist	NASH	NCT02854605	Phase II
Tropifexor (LJN452)	FXR agonist	NASH	NCT02855164	Phase II
TERN-101	FXR agonist	NASH	NCT04328077	Phase II
EDP-305	FXR agonist	NASH	NCT03421431	Phase II
EYP001a	FXR agonist	NASH	NCT03812029	Phase II
LMB763	FXR agonist	NASH	NCT02913105	Phase II
Exenatide	GLP-1 agonist	NAFLD	NCT01208649	Phase IV
Liraglutide	GLP-1 agonist	NASH, NAFLD	NCT02654665	Phase III
Semaglutide	GLP-1 agonist	NASH	NCT04822181	Phase III
Dulaglutide	GLP-1 receptor agonist	T2DM, NASH	NCT03648554	Phase IV
Tirzepatide	Dual GIP and GLP-1 receptor agonist	NASH	NCT04166773	Phase II
BI456906	Dual GIP and GLP-1 receptor agonist	NASH	NCT04771273	Phase II
Sitagliptin	DPP4 inhibitor	NAFLD	NCT01963845	Phase II
Evogliptin	DPP4 inhibitor	NAFLD, T2DM	NCT03910361	Phase IV
LIK066	SGLT1/2 inhibitor	Obese patients with NASH	NCT03205150	Phase II
Empagliflozin	SGLT2 inhibitor	NAFLD, T2DM	NCT02964715	Phase IV
Ipragliflozin	SGLT2 inhibitor	NAFLD, T2DM	NCT02875821	Phase IV
Dapagliflozin	SGLT2 inhibitor	NASH	NCT03723252	Phase III
Canagliflozin	SGLT2 inhibitor	NAFLD with T2DM	NCT05513729	Phase I
Ertugliflozin	SGLT2 inhibitor	NAFLD/NASH with liver fat, liver fibrosis, T2DM	NCT05644717	Phase IV
Resmetirom (MGL-3196)	THR-β Agonist	NASH	NCT03900429	Phase III
VK2809	THR-β Agonist	Hyperlipidemia, NAFLD	NCT02927184	Phase II
NGM282	FGF19 agonist	NASH	NCT03912532	Phase II
Oltipraz	Liver X receptor alpha (LXR-α)	NAFLD	NCT04142749	Phase III
Gemcabene	APOC3 inhibitor	NAFLD	NCT03436420	Phase II (terminated due to lack of efficacy and safety concerns)
Anti-inflammation				
JKB-121	TLR-4 antagonist	NASH	NCT02442687	Phase II
Cenicriviroc	CCR2/5 antagonist	NASH	NCT03028740	Phase III (terminated due to lack of efficacy)
BI 1467335	Amine oxidase copper containing 3 (AOC3) inhibitor	NAFLD	NCT03166735	Phase II
Namodenoson	Adenosine receptor agonist	NASH	NCT04697810	Phase II
IMM-124E	Anti-LPS	NASH	NCT02316717	Phase II

(Continued)

TABLE 3 Continued

Medication	Primary mechanism	Inclusion criteria	Clinical trial number	Trial phase
Solithromycin	Anti-LPS	NASH	NCT02510599	Phase II
Anti-fibrosis				
Pegbelfermin	FGF21 agonist	NASH with stage 3 fibrosis	NCT03486899	Phase II
Pegbelfermin	FGF21 agonist	NASH with cirrhosis, NAFLD, Liver fibrosis	NCT03486912	Phase II (completed)
Efruxifermin	FGF21 agonist	NASH	NCT03976401	Phase II
MK-3655	Monoclonal antibody agonist of the b-Klotho/FGFR1c receptor complex	NASH	NCT04583423	Phase II
Belapectin (GR-MD-02)	Galectin-3 inhibitor	NASH	NCT04365868	Phase III
Losartan	Angiotensin II receptor blocker	NASH	NCT01051219	Phase III
Simtuzumab (SIM)	LOXL2 monoclonal antibody	NASH	NCT02466516	Phase II
ND-L02-s0201 (BMS-986263)	HSP47 siRNA	NASH	NCT04267393	Phase II
MT-3995	Mineralocorticoid receptor antagonist	NASH	NCT02923154	Phase II
AZD2693	Patatin like phospholipase domain containing 3 (PNPLA3) inhibitor	NASH	NCT04483947	Phase I
CC-90001	JNK inhibitor	NASH with Stage 2, Stage 3 liver fibrosis	NCT04048876	Phase II (terminated due to changes in business objectives)
Anti-apoptosis				
Selonsertib	ASK1 inhibitor	NASH	NCT03053063	Phase III (terminated due to lack of efficacy)
Emricasan (ENCORE-NF)	Caspase Inhibitor	NASH, Fibrosis, liver disease	NCT02686762	Phase II
Antioxidants				
Vitamin E	Antioxidants	NAFLD, NASH	NCT04801849	Phase II
Pentoxifylline	Phosphodiesterase inhibitor	NASH	NCT05284448	Phase III

8 Conclusion

Obesity is a highly prevalent and recurrent disease that increases the risk of HCC by exacerbating the onset and progression of NAFLD and NASH. Extensive studies have focused on understanding the pathophysiology of NAFLD/NASH and obesity-associated HCC, including the molecular mechanisms and related signaling pathways. A diversity of preclinical experimental animal models have been developed to facilitate *in vivo* research, each with advantages and limitations depending on the research hypothesis. More recently non-invasive technologies such as serum biomarkers have been developed for the early diagnosis of NAFLD/NASH. Numerous candidate drugs have exhibited efficacy in fibrosis, inflammation and steatosis in clinical trials. These findings may open up novel approaches to treatment. There remains an unmet need for reliable biomarkers and non-invasive tools to accurately stage the progression of NAFLD/NASH and to validate the safety and efficacy of potential therapies in clinical trials. Furthermore, a personalized medicine approach will be needed to tailor the right therapeutic approach to optimize the treatments in individual patients for obesity-related liver disease.

Author contributions

YC and SA discussed the content, YC wrote the first draft of the manuscript. All the listed authors reviewed and revised the text. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Association between diabetes status and breast cancer in US adults: findings from the US National Health and Nutrition Examination Survey

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Objectives: The aim of this study was to investigate the association between diabetes status and the risk of breast cancer among adult Americans, exploring the impact of BMI, age, and race on this relationship.

Methods: A cross-sectional analysis of 8,249 individuals from the National Health and Nutrition Examination Survey (NHANES) was conducted. Diabetes was categorized as type 2 diabetes and prediabetes, with both conditions diagnosed according to the ADA 2014 guidelines. The association between diabetes status and breast cancer risk was explored using multiple logistic regression analysis.

Results: Patients with diabetes had higher odds of breast cancer (OR: 1.51; 95% CI 1.00 to 2.28), Using the two-piecewise linear regression model, it was observed that there is a threshold effect in the risk of breast cancer occurrence at the age of 52 years. Specifically, the risk of breast cancer is relatively low before the age of 52 but increases significantly after this age.

Conclusions: This study identified a significant association between diabetes status and breast cancer risk among adult Americans. We also found a threshold effect in breast cancer occurrence at the age of 52. Age was significantly

associated with breast cancer risk in both Non-Hispanic White and Non-Hispanic Black individuals. These findings underscore the importance of diabetes management, maintaining a healthy BMI, and age-related risk considerations in reducing breast cancer risk.

KEYWORDS

diabetes status, prediabetes, type 2 diabetes, obesity, breast cancer, NHANES

Introduction

There are more than 40 million cases of breast cancer in women worldwide and it is the second most common cancer among women in the United States (1, 2). The American Cancer Society indicates that approximately 42,000 women will die from breast cancer in 2020, with 276,000 newly diagnosed cases (3). Breast cancer affects women of all ages. However, the incidence of breast cancer increases with age, with a peak incidence at 45-64 years (4). There are many factors associated with the risk of breast cancer (5, 6). The prevalence of diabetes is increasing at an alarming rate and has become one of the most serious public health problems in the world. Diabetes is also considered to be the most common endocrine disease. The American Diabetes Association (ADA) shows that diabetes is the fourth leading cause of death in the United States (7).

There is a growing recognition that type 2 diabetes mellitus (T2DM) and breast cancer (BC) occur together in the same patient population with high mortality rates (8). Overall survival and disease-specific survival are significantly worse in diabetic BC patients compared to non-diabetic BC patients, suggesting a correlation between T2DM and cancer progression (9). Hardefeldt et al. showed that diabetes mellitus is an independent risk factor for breast cancer (10). According to the results of a meta-analysis, women with diabetes had a 23% higher risk of future breast cancer than women without diabetes (11). A meta-analysis showed that women with diabetes had a significantly higher risk (~20%) of breast cancer than those without diabetes (12). T2DM and hyperinsulinemia were independently associated with postmenopausal breast cancer (13). In addition, a growing body of data suggests that diabetes and its complications adversely affect cancer treatment (14) and increase mortality (15), thereby affecting the prognosis of breast cancer patients (16, 17). Studies have suggested that the higher risk of breast cancer among the diabetes patients can be resulted from detection bias or potential confounders (18, 19); and that the use of antidiabetic drugs might affect the risk of breast cancer.

Patients with prediabetes have higher than normal blood glucose levels, but not high enough to be considered asT2DM. However, this is often seen as a warning sign. Prediabetes is characterized by impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or an HbA1c of 39 mmol/mol (5.7%) to 46 mmol/mol (6.4%) (20). The significance of prediabetes lies in the risk associated with progression to T2DM, which is disproportionately higher at the upper end of the prediabetes range and in the combined presence of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) (20). Prediabetes and T2DM are parts of a continuum of spectrum that share pathophysiology and are associated with typical phenotypes including obesity, hypertension (HTN) and dyslipidemia (DLP) (21). Despite extensive research on the association between diabetes and breast cancer, many aspects of the relationship and underlying mechanisms remain unclear. Therefore, further research in this area is necessary.The aim of this study was to investigate the relationship between diabetes status and breast cancer in United States adults using data from the National Health and Nutrition Examination Survey (NHANES) from 2011-2016. Specifically, the objectives of this study were to: 1) examine the distribution of diabetes status (T2DM, prediabetes, and non-diabetes) in the study population; 2) determine the correlation between diabetes status and breast cancer; 3) determine the relationship between race and breast cancer; and 4) determine the relationship between BMI and breast cancer. By analyzing these factors, we aimed to gain a better understanding of the risk factors associated with breast cancer in relation to diabetes status.

Materials and methods

Data source

NHANES is a cross-sectional, population-based survey that assesses the health and nutritional status of the United States civilian, noninstitutional population through interviews, physical examinations, and laboratory tests. It is publicly available, and data is released every two years on a nationally representative sample using a multistage probability sampling design and weights (22). The NHANES program is reviewed annually by the National Center for Health Statistics Ethics Review Committee to ensure its ethical and scientific standards (23).

Study population

The data used in this study were obtained from the 2011-2016 survey cycle (24). This provides information on all the variables that have been used to determine the risk factors and determinants of type 2 diabetes in recent years. The process for study selection is

shown in the flow diagram in Figure 1. Multiple interpolation was used for missing data.

Diagnostic criteria for diabetes and prediabetes

The diagnostic criteria for type 2 diabetes mellitus (T2DM) and prediabetes are shown in Supplement Table 1 and the study population had to meet the diagnostic criteria or have a clear diagnosis of diabetes in NHANES.

Statistical analysis

Data were presented as weighted mean ± standard error (SE) for continuous variables and weighted percentages (95% confidence interval) for categorical variables. The associations between diabetes status and breast cancer, as well as race and breast cancer, were examined using logistic regression models. Three models were employed for the analysis: Model 1 as the crude model with no adjustments, Model 2 adjusted for age, race, and body mass index (BMI), and Model 3 adjusted for age, race, BMI, educational level, serum creatinine, cholesterol, triglycerides, glycohemoglobin, serum cotinine, estradiol, marital status, serum glucose, and reproductive health. The threshold effect analysis of BMI and age on breast cancer was assessed using two-piecewise linear regression models. The inflection points for BMI and age were determined, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for both below and above these inflection points. The log-likelihood ratio was also reported to evaluate the goodness-of-fit of the models. Additionally, the threshold effect analysis of BMI and age for different racial groups was performed using the standard linear model, and the adjusted ORs with 95% CIs were reported for each racial group. The statistical software package R (http://www.R-project.org) was used for statistical analyses. Statistical significance was considered when the P value was < 0. 05.

Results

Characteristics of the participants

Table 1 presents the weighted characteristics of the study sample, which consisted of 8,249 participants classified by NHANES and grouped by diabetes status (Type 2 diabetes, prediabetes, and non-diabetes). Our findings showed that the Type 2 diabetes group had a significantly higher BMI (33.934 kg/m2) than both the Prediabetes group (30.988 kg/m2) and Non-diabetes group (27.660 kg/m2) ($p < 0.0001$). Additionally, there was no significant difference in serum nicotine levels between the Type 2 diabetes group and the Non-diabetes group ($p = 0.625$). However, the estradiol level in the Type 2 diabetes group was significantly lower (35.884 pg/mL) than the Prediabetes group (69.905 pg/mL) and Non-diabetes group (142.538 pg/mL) ($p < 0.0001$). Furthermore, the age of menarche in the Type 2 diabetes group (12.534 years) was significantly lower than the Prediabetes group (12.751 years) and Non-diabetes group (12.774 years) ($p < 0.001$). Lastly, the age of menopause in the Type 2 diabetes group (42.751 years) was significantly higher than the Prediabetes group (41.533 years) and Non-diabetes group (35.999 years) ($p < 0.0001$). More detailed results can be found in Table 1.

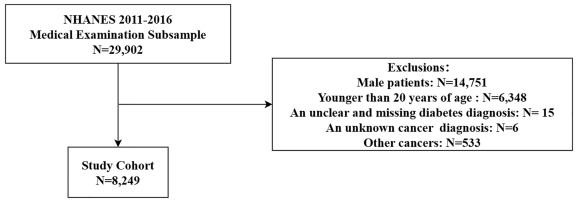

FIGURE 1
Research flowchart.

Associations between diabetes status and breast cancer

Table 2 shows the results of multiple logistic regression analysis of the association between diabetes status (non-diabetes, prediabetes, and Type 2 diabetes) and breast cancer, with odds ratios (ORs) and 95% confidence intervals (CIs) for three different models. Model 1 does not adjust for any covariates, Model 2 adjusts for age, race, and body mass index (BMI), and Model 3 adjusts for age, race, BMI, educational level, serum creatinine, cholesterol, triglycerides, glycohemoglobin, serum cotinine, estradiol, marital status, serum glucose, and reproductive health. For the non-diabetes group, the ORs in all three models are considered the reference group. For the prediabetes group, the OR in Model 1 is 1.57 (95% CI, 1.13-2.16, P=0.006), in Model 2 is 0.92 (95% CI, 0.66-1.28, P=0.627), and in Model 3 is 0.90 (95% CI, 0.64-1.26, P=0.530). For the Type 2 diabetes group, the OR in Model 1 is 2.99 (95% CI, 2.21-4.05, P<0.0001), in Model 2 is 1.63 (95% CI, 1.18-2.26, P=0.003), and in Model 3 is 1.51 (95% CI, 1.00-2.28, P=0.049). Overall, these findings suggest that Type 2 diabetes is significantly associated with an increased risk of breast cancer, even after adjusting for multiple covariates. Results are detailed in Table 2.

Associations between prediabetes/diabetes and breast cancer by race

Table 3 shows the results of multiple logistic regression analysis testing the relationship between race and breast cancer. The unadjusted model (Model 1) was first examined, followed by Model 2 adjusted for age and body mass index, and finally Model 3 adjusted for additional covariates, including educational level, serum creatinine, cholesterol, triglycerides, glycohemoglobin, serum cotinine, estradiol, marital status, serum glucose, and reproductive health. For each racial group and diabetes status, the odds ratios (ORs) with 95% confidence intervals (CIs) and p-values were calculated, with the non-diabetes group as the reference. For

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TABLE 1 Weighted characteristic of study sample.

	Type 2 diabetes	Prediabetes	Non-diabetes	P-value
N	1452	2105	4692	
Body mass index (kg/m^2)	33.934 (0.288)	30.988 (0.264)	27.660 (0.150)	< 0.0001
Cholesterol (mmol/L)	4.961 (0.041)	5.281 (0.033)	4.998 (0.019)	< 0.0001
Creatinine (umol/L)	78.413 (1.720)	69.420 (0.971)	66.153 (0.374)	< 0.0001
Serum glucose (mmol/L)	7.999 (0.124)	5.452 (0.022)	4.923 (0.015)	< 0.0001
Triglycerides (mmol/L)	2.110 (0.079)	1.611 (0.031)	1.350 (0.021)	< 0.0001
Serum Cotinine (ng/mL)	38.499 (3.590)	42.957 (3.337)	39.828 (2.471)	0.625
Estradiol (pg/mL)	35.884 (2.278)	69.905 (9.925)	142.538 (8.822)	< 0.0001
Glycohemoglobin (%)	7.049 (0.055)	5.686 (0.011)	5.242 (0.008)	< 0.0001
Age when first menstrual period occurred	12.534 (0.055)	12.751 (0.057)	12.774 (0.027)	< 0.001
Age at last menstrual period	42.751 (0.262)	41.533 (0.219)	35.999 (0.267)	< 0.0001
Age (years)				< 0.0001
≤45	257 (19.240)	638 (30.515)	2959 (62.474)	
>45	1195 (80.760)	1467 (69.485)	1733 (37.526)	
Race				
Non-Hispanic White	400 (55.204)	727 (64.216)	1759 (64.813)	< 0.0001
Non-Hispanic Black	422 (17.934)	518 (13.471)	984 (11.544)	
Non-Hispanic Asian	149 (5.894)	279 (6.160)	650 (5.967)	
Mexican American	243 (10.586)	290 (7.571)	596 (8.335)	
other	238 (10.381)	291 (8.581)	703 (9.340)	
Education level				< 0.0001
Less than 9th grade	256 (10.735)	237 (5.944)	311 (3.777)	
9-11th grade	231 (13.030)	264 (10.662)	514 (7.943)	
High school graduate/GED or equivalent	342 (25.544)	434 (20.415)	934 (18.726)	
Some college or AA degree AA degree	417 (34.004)	654 (32.806)	1577 (35.067)	
College graduate or above	206 (16.687)	516 (30.173)	1356 (34.487)	
Marital status				< 0.0001
Married	636 (49.564)	1001 (52.982)	2157 (51.096)	
Widowed	293 (17.923)	273 (11.529)	304 (4.736)	
Divorced	207 (13.276)	303 (13.750)	501 (10.955)	
Separated	76 (3.592)	99 (3.269)	155 (2.519)	
Never married	181 (11.437)	284 (11.362)	1129 (21.441)	
Living with partner	59 (4.207)	145 (7.108)	446 (9.253)	
Ever been pregnant				< 0.0001
no	137 (11.212)	224 (12.186)	948 (23.179)	
yes	1315 (88.788)	1881 (87.814)	3744 (76.821)	
Breast cancer				< 0.0001
no	1370 (94.187)	2041 (96.707)	4600 (97.930)	
yes	82 (5.813)	64 (3.293)	92 (2.070)	

Continuous variables were expressed as weighted mean ± standard error (SE).
Categorical variables were expressed as weighted percentages (95% confidence interval).

TABLE 2 Associations between diabetes status and breast cancer.

	Model 1	Model 2	Model 3
	OR (95% CI, P)	OR (95% CI, P)	OR (95% CI, P)
Non- diabetes	Reference	Reference	Reference
Prediabetes	1.57 (1.13,2.16) P=0.006	0.92 (0.66, 1.28) P=0.627	0.90 (0.64, 1.26) P=0.530
Type 2 diabetes	2.99 (2.21,4.05) P<0.0001	1.63 (1.18, 2.26) P=0.003	1.51 (1.00, 2.28) P=0.049

Model 1: Adjust for: None.
Model 2: Age, race, body mass index were adjusted.
Model 3: Age, race, body mass index, educational level, serum creatinine, cholesterol, triglycerides, glycohemoglobin, serum cotinine, estradiol, marital status, serum glucose and reproductive health were adjusted.

example, Model 1 showed that among non-Hispanic White individuals, those with type 2 diabetes had an increased risk of breast cancer, with an OR of 2.92 (95% CI, 1.87-4.49, P < 0.0001). The results are shown in Table 3.

Analysis of the effect of BMI threshold on female breast cancer using a two-part linear regression model

Table 4 displays the results of a threshold effect analysis examining the relationship between body mass index (BMI) and breast cancer risk in women using a two-piecewise linear regression model. The adjusted odds ratios (ORs) with 95% confidence intervals (CIs) are presented. The table compares the results of fitting the standard linear model with those of the two-piecewise linear model. The inflection point is at 21 kg/m2. For individuals with BMI less than 21 kg/m2, the adjusted OR for breast cancer is 0.88 (95% CI: 0.69, 1.11). For individuals with BMI greater than 21 kg/m2, the adjusted OR for breast cancer is 1.01 (95% CI: 0.98, 1.03). The log-likelihood ratio is 0.297. Results are detailed in Table 4; Figure 2. Figure 3 shows the relationship between BMI and breast cancer among different racial/ethnic groups. These findings suggest that there may be a threshold effect of BMI on breast cancer risk in women.

Threshold effect analysis of age on breast cancer in female using the two piecewise linear regression model

Table 5 presents the results of the threshold effect analysis of age on breast cancer in females using the two-piecewise linear regression model. The table shows the adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for both the standard linear model and the two-piecewise linear model. The standard linear model yielded an adjusted OR of 1.08 (95% CI: 1.06, 1.09). However, the two-piecewise linear model identified an inflection point at age 52 years. Among females aged less than 52 years, the adjusted OR was 1.18 (95% CI: 1.12, 1.26), while for those aged over 52 years, the adjusted OR was 1.06 (95% CI: 1.04, 1.08). The log-likelihood ratio was less than 0.001, indicating that the two-piecewise linear model was a better fit for the data than the standard linear model. These findings suggest that age has a threshold effect on the risk of breast cancer in females, with the risk increasing significantly after age 52 years. The results are presented in Table 5; Figure 4. Figure 5 shows the relationship between Age and breast cancer among different racial/ethnic groups.

Threshold effect analysis of BMI/Age using the standard linear model across different racial/ethnic groups

Table 6 presents the results of the threshold effect analysis of BMI/age using the standard linear model for different racial/ethnic groups. The adjusted odds ratios (ORs) with 95% confidence intervals (CIs) and p-values are shown for each group. For Non-Hispanic White individuals, the ORs for BMI and age were 0.99 (95% CI, 0.96-1.03, P=0.7253) and 1.08 (95% CI, 1.05-1.10, P<0.0001), respectively. Similarly, for Non-Hispanic Black individuals, the ORs were 1.01 (95% CI, 0.97-1.05, P=0.6611) for BMI and 1.08 (95% CI, 1.04-1.12, P=0.0001) for age. For Non-Hispanic Asian individuals, the ORs were 1.01 (95% CI, 0.90-1.13, P=0.8865) for BMI and 1.12 (95% CI, 1.05-1.19, P=0.0005) for age. For Mexican American individuals, the ORs were 1.06 (95% CI, 0.99-1.13, P=0.1022) for BMI and 1.09 (95% CI, 1.03-1.14, P=0.0016) for age. For individuals from other racial/ethnic groups, the ORs were 0.96 (95% CI, 0.90-1.02, P=0.2252) for BMI and 1.06 (95% CI, 1.02-1.10, P=0.0031) for age. The results indicate that the association between BMI/age and breast cancer risk varies across different racial/ethnic groups. Table 6; Figures 3, 5 display the results.

Discussion

The present study investigated the associations between diabetes status, BMI, age, and breast cancer risk in a representative sample of US adults, using data from the NHANES. Our analysis revealed significant relationships between diabetes status, BMI, and age with breast cancer risk, with varying associations observed across different racial groups. Our results demonstrated that individuals with Type 2

TABLE 3 Associations between prediabetes/diabetes and breast cancer by race.

	Model 1	Model 2	Model 3
	OR (95% CI, P)	OR (95% CI, P)	OR (95% CI, P)
Non-Hispanic White			
Non- diabetes	Reference	Reference	Reference
Prediabetes	1.35 (0.86,2.10) P=0.184	0.82 (0.51, 1.29) P=0.401	0.82 (0.50, 1.30) P=0.399
Type 2 diabetes	2.92 (1.87,4.49) P<0.0001	1.64 (1.02, 2.61) P=0.039	1.46 (0.79, 2.64) P=0.215
Non-Hispanic Black			
Non- diabetes	Reference	Reference	Reference
Prediabetes	1.92 (0.82,4.51) P=0.129	1.05 (0.44, 2.52) P=0.910	1.12 (0.45,2.81) P=0.801
Type 2 diabetes	3.71 (1.74,8.24) P<0.001	1.76 (0.79, 4.09) P=0.170	2.18 (0.78,6.23) P=0.140
Non-Hispanic Asian			
Non- diabetes	Reference	Reference	Reference
Prediabetes	1.96 (0.56, 6.56) P=0.270	0.96 (0.27,3.25) P=0.941	0.98 (0.25,3.75) P=0.971
Type 2 diabetes	6.09 (2.09,18.76) P<0.001	2.27 (0.73,7.31) P=0.155	3.29 (0.66,1.60) P=0.138
Mexican American			
Non- diabetes	Reference	Reference	Reference
Prediabetes	2.79 (0.96, 8.54) P=0.060	1.25 (0.42,3.91) P=0.682	0.94 (0.29, 3.11) P=0.915
Type 2 diabetes	3.78 (1.35,11.39) P=0.013	1.24 (0.43,3.87) P=0.693	5.30 (0.10, 2.49) P=0.433
Other			
Non- diabetes	Reference	Reference	Reference
Prediabetes	1.69 (0.69,3.97) P=0.230	0.99 (0.40, 2.39) P=0.987	0.90 (0.35, 2.23) P=0.824
Type 2 diabetes	3.07 (1.39,6.77) P=0.005	1.72 (0.74, 3.99) P=0.204	1.43 (0.46, 4.19) P=0.523

Model 1: Adjust for: None.
Model 2: Age, body mass index were adjusted.
Model 3: Age, body mass index, educational level, serum creatinine, cholesterol, triglycerides, glycohemoglobin, serum cotinine, estradiol, marital status, serum glucose and reproductive health were adjusted.

TABLE 4 Threshold effect analysis of BMI on breast cancer in female using the two piecewise linear regression model.

Breast cancer	Adjusted OR (95% CI)
Fitting by the standard linear model	1.00 (0.98, 1.02)
Fitting by the two-piecewise linear model	
Inflection point	21
Body mass index (kg/m^2) < 21 (kg/m^2)	0.88 (0.69, 1.11)
Body mass index (kg/m^2) > 21 (kg/m^2)	1.01 (0.98, 1.03)
Log likelihood ratio	0.297

diabetes had a significantly higher risk of breast cancer compared to those without diabetes. This association persisted even after adjusting for multiple covariates, such as age, race, BMI, and other potential confounders. These findings are in line with previous research indicating that Type 2 diabetes may be associated with an increased risk of breast cancer (25). Possible explanations for this relationship include hyperinsulinemia, insulin resistance, and chronic inflammation, which have been suggested to contribute to breast cancer development and progression (26). In addition, our study showed that individuals with prediabetes had no significant increase in breast cancer risk compared to those without diabetes. This finding emphasizes the need for further research to understand the role of

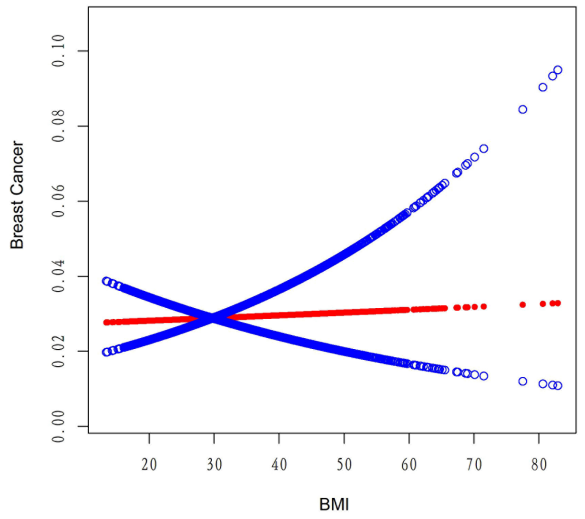

FIGURE 2
The association between BMI and breast cancer risk.

glycemic control and potential interventions to reduce breast cancer risk among individuals with diabetes.

Our threshold effect analysis revealed an inflection point at 21 kg/m² in the relationship between BMI and breast cancer risk. For individuals with a BMI greater than 21 kg/m², the risk of breast cancer increased, whereas those with a BMI less than 21 kg/m² had no significant change in risk. These findings are consistent with previous research demonstrating that higher BMI is associated with an increased risk of postmenopausal breast cancer (27). Several mechanisms have been proposed to explain this relationship, including increased estrogen production in adipose tissue, altered adipokine and insulin signaling, and increased inflammation (28). Our analysis also identified a threshold effect of age on breast cancer risk, with a significant increase in risk observed after the age of

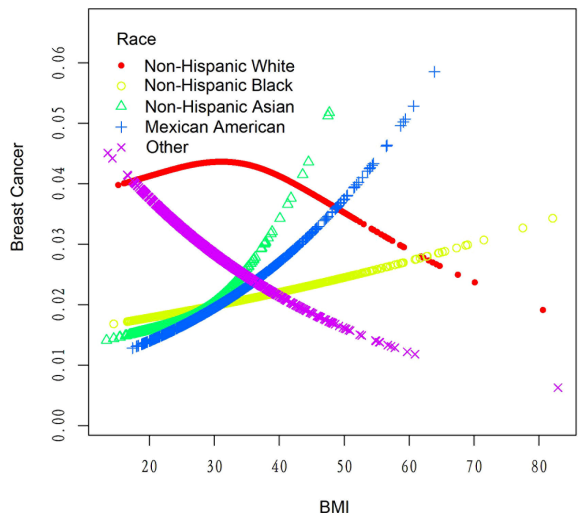

FIGURE 3
The association between BMI and breast cancer risk among different populations.

TABLE 5 Threshold effect analysis of age on breast cancer in female using the two piecewise linear regression model.

Breast cancer	Adjusted OR (95% CI)
Fitting by the standard linear model	1.08 (1.06, 1.09)
Fitting by the two-piecewise linear model	
Inflection point	52
age (years) < 52 (years)	1.18 (1.12, 1.26)
age (years) > 52 (years)	1.06 (1.04, 1.08)
Log likelihood ratio	<0.001

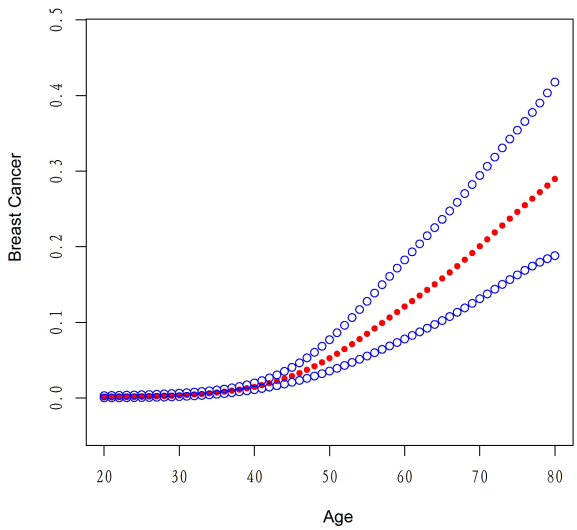

FIGURE 4
The association between age and breast cancer risk.

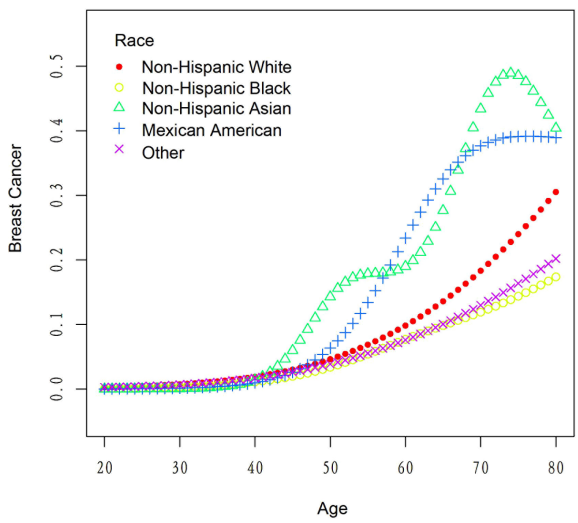

FIGURE 5
The association between age and breast cancer risk among different populations.

TABLE 6 Threshold effect analysis of BMI/Age using the standard linear model.

	BMI		Age	
	Adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Non-Hispanic White	0.99 (0.96, 1.03)	0.7253	1.08 (1.05, 1.10)	<0.0001
Non-Hispanic Black	1.01 (0.97, 1.05)	0.6611	1.08 (1.04, 1.12)	0.0001
Non-Hispanic Asian	1.01 (0.90, 1.13)	0.8865	1.12 (1.05, 1.19)	0.0005
Mexican American	1.06 (0.99, 1.13)	0.1022	1.09 (1.03, 1.14)	0.0016
other	0.96 (0.90, 1.02)	0.2252	1.06 (1.02, 1.10)	0.0031

52 years. This finding is in line with existing literature, which has consistently reported that breast cancer risk increases with age, particularly after menopause (29). The increased risk at older ages may be attributed to the accumulation of genetic and epigenetic changes over time, as well as age-related changes in hormone levels and immune function (30).

In our study, we discovered that the relationships between BMI, age, and breast cancer risk exhibited variations across different racial groups. However, it is important to note that the differences in the association between BMI and breast cancer risk among various racial groups were not statistically significant. This finding highlights the complexity of the relationship between BMI and breast cancer risk, and suggests that further research is necessary to better understand the underlying factors that may contribute to these variations, such as differences in body fat distribution, hormone levels, and genetic factors (31). On the other hand, age was found to be significantly associated with breast cancer risk across all racial groups, emphasizing the importance of age as a universal risk factor for breast cancer (32).

Our study has several limitations that should be considered when interpreting the findings. First, the cross-sectional nature of the data precludes establishing causal relationships between diabetes status, BMI, age, and breast cancer risk. Longitudinal studies are needed to confirm these associations and investigate potential underlying mechanisms. Second, the reliance on self-reported data may introduce recall bias, particularly for variables such as age at menarche and age at menopause. Future studies could benefit from objective measures to minimize potential biases. Third, although we adjusted for multiple covariates, residual confounding cannot be ruled out. There may be additional unmeasured factors, such as genetic predisposition, environmental exposures, and lifestyle factors, that contribute to the observed associations.

Despite these limitations, our study provides valuable insights into the relationships between diabetes status, BMI, age, and breast cancer risk in a diverse US population. Our findings highlight the importance of considering these factors in breast cancer prevention strategies and suggest that targeted interventions for individuals with Type 2 diabetes may be beneficial in reducing breast cancer risk. Moreover, our results underscore the need for further research to understand the mechanisms underlying the associations between diabetes status, BMI, age, and breast cancer risk, as well as the potential differences in these relationships across racial groups.

Future research should aim to replicate our findings in larger, prospective cohorts and investigate the biological pathways linking diabetes, obesity, and age to breast cancer development. Additionally, intervention studies targeting glycemic control, weight management, and other modifiable risk factors could help determine the effectiveness of such strategies in reducing breast cancer risk among individuals with diabetes and those with higher BMI. Finally, understanding the racial differences in the relationships between these factors and breast cancer risk may contribute to the development of more targeted and effective prevention strategies for different populations.

Conclusion

In conclusion, our study demonstrates significant associations between diabetes status, BMI, age, and breast cancer risk in a representative US population. These findings highlight the importance of considering these factors in breast cancer prevention efforts and suggest that targeted interventions may be warranted to reduce breast cancer risk among individuals with Type 2 diabetes and those with higher BMI. Further research is needed to elucidate the underlying mechanisms and identify effective prevention strategies for diverse populations.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

XS, QZ, and KK: They were responsible for drafting the work and critically revising it for important intellectual content. PH, XL, JL, YY, CS, VY, SL, and MM: They contributed to the acquisition, analysis, and interpretation of data for the work. ZL and MZ. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1059303/full#supplementary-material

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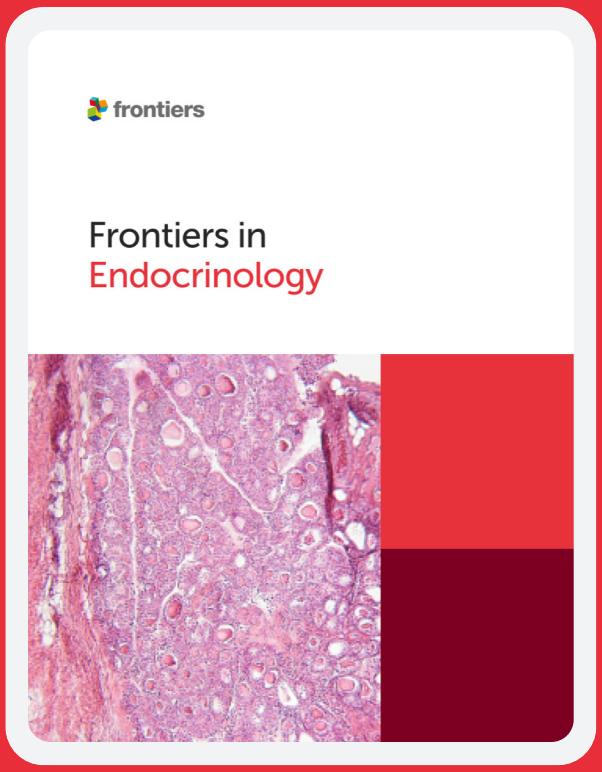

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