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Modeling Human Viral Diseases: Trials and Triumphs

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Understanding and studying human diseases caused by pathogens require tools other than the natural host, as many diseases are lethal to humans. To understand the mechanism(s) involved in pathogenesis caused either by genetics or microbes including viruses, it is important to have model systems where disease can be induced by altering genes or host-pathogen interactions and where the functional consequences can be monitored closely. Among the human pathogens, viruses are known to cause debilitating outbreaks resulting in significant morbidity and mortality due to the emerging and/or reemerging outbreaks (1–3). Many virus species are associated with human diseases ranging from hemorrhagic, gastroenteric, pharyngeal to neuroinvasion (4–10). Thus, understanding the virus biology and their ability to establish infection and induce pathogenesis in human host remains a priority. However, studying pathogenic viruses require appropriate host species other than humans to model diseases development and progression. For centuries, researchers have used animal models inoculated with viruses for the purpose of monitoring and manipulating disease progression. In this way, animal models have contributed significantly to not only understanding disease development, but also to defining vaccine efficacy, and to drug and treatment development (11–14). However, the availability of larger animals (both inbreed and out breed), cost and ethical concerns pushed the field to identify alternate model systems to study virus-host interactions. More recently, 2D cell culture and 3D organoid models have become increasingly prominent and refined disease models for dissecting the molecular mechanisms underlying viral induced pathogenesis (14–17). Each model has strengths and weaknesses and understanding how these relate to disease phenotypes is key to interpreting the results obtained from these systems.

ANIMAL MODELS FOR VIRAL DISEASES

One of the most common approaches to study virus infection and the associated disease development is to use small and large animal models that recapitulate human diseases. For example, studies on virus-host interaction and pathogenesis were modeled using rodent, rabbits, dogs, horses, Ferrets, and non-human (18–22). The major advantage of using the animal model is the presence of immune system which mimics the effect of immune response during disease development. Furthermore, in some animal models the immune system could be manipulated in ways that makes it possible to ascertain the contribution of specific immune components to disease outcome (23, 24). Mice, rats, Ferrets and non-human primates are also used to study pathogenesis induced by viruses, bacteria, and parasites as well as in vaccine efficacy studies (22, 25–27), but cross-model interpretations are limited by the fact that these animal models may not represent similar disease pathogenesis due to inter species variation and genetic background. For instance, requirements of species-specific entry receptors for HBV, SARS-CoV-2, HIV, and other process are key for infection and disease development (28, 29). Additionally, animal models are oftentimes infected with a species-specific counter part of a human pathogen (e.g., SIV vs. HIV) that corresponds to the receptors and proteins necessary for transmission and disease development

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in that host. These differences in physiology necessitated the development of transgenic animals with human specific cell types that will serve as target cell for human pathogens with appropriate receptors (23, 24). Significant progress has been made in this area of research; however, there are several restrictions, including the presence of species specific innate immune factors that hampered the infection, spread, and transmission within these animals (21, 30). More importantly, the downstream and secondary effects caused by viruses, viral proteins and its byproducts can't be fully studied using these models. Additionally, animal models, either small or large, are expensive, labor intensive, and subject to the consideration of ethical issues, especially for work involving non-human primates and humanized mice.

IN VITRO CELL CULTURE MODEL TO STUDY VIRAL INFECTION

Cell culture models have become increasingly valuable research tools and studies using 2D single cell lineages infected with pathogens have significantly expanded our understanding the biology of pathogens. For example, single cell-based culture models provided a unique opportunity to study how viruses (HCV, hemorrhagic fever viruses, HIV) induce cellular dysregulation and pathogenic effects in specific cell types (16, 17, 31). Specific studies using primary cell based 2D cultures offered a great avenue to understand the changes at the cellular level and more precisely at the cellular level (17). These studies furthered our attempts to identify the specific target cell types involved in disease development, the requirements of cell-specific receptors, gene control, and the ability of pathogens to manipulate host cellular genes and signaling pathways. However, in the body, cells function as part of a network of cells and a lack of holistic to integrate additional cells in 2D cultures, including other cell types within a specific organ, reduce the physiologic relevance and significance of this system. Additionally, the innate defense responses induced by other cell types in the surrounding microenvironment is lacking in 2D cell culture models. Thus, developing 3D organoid models that mimic *in vivo* conditions for studying normal development and differentiation and disease pathologies has become a priority. Several approaches have been taken to create these models ranging from co-culturing multiple cells within a tissue culture well to development of 3D organoids that represent different organ systems to study respiratory and neurotropic viruses (26, 32, 33).

Organoids using supporting materials such as Matrigel and other scaffolding elements lead to 3D organoids where cells differentiate and mature into various cell types within a specific organ. Several 3D organoid models have been successfully established including models of brain and/or cerebral, intestine/colon and retina. Importantly, 3D organoids have great potential for modeling viral diseases including Zika, Influenza, HIV-, and others (34, 35). More recently organ specific human tissues and tissue-based organoids (lung, heart, and brain) are used to study influenza, SARS-CoV-2, Zika, and Dengue virus infection and pathogenesis (36–38). Though the organoid and tissue models provide a great *in vitro* alternate model that is quick and in expensive, there are certain limitations associated with them as well. For instance, maintaining these cultures for a longer period to study chronic viral diseases is an issue. This is due to lack vascularization in this model, that will prevent the flow of nutrients to the core region of the 3D-organoid structures.

Studying host-pathogen including infectious viruses that cause morbidity and mortality in humans is a challenging and constantly evolving area of research. The recent emergence and reemergence of pathogenic viruses force the scientific community to find alternate *in vitro* and *in vivo* systems that will provide a suitable platform to model viral pathogenesis and disease development. As indicated above, there are several models that have both pros and cons, however, a great deal of progress has been made to model several viral diseases. With constant addition and/or reinvention, these models can be tweaked to mimic physiological relevance of the host, that will help us to study viral diseases and develop therapeutics and vaccines.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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