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Mini-review

Viral infections and breast cancer – A current perspective

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ABSTRACT

Sporadic human breast cancer is the most common cancer to afflict women. Since the discovery, decades ago, of the oncogenic mouse mammary tumour virus, there has been significant interest in the potential aetiologic role of infectious agents in sporadic human breast cancer. To address this, many studies have examined the presence of viruses (e.g. papillomaviruses, herpes viruses and retroviruses), endogenous retroviruses and more recently, microbes, as a means of implicating them in the aetiology of human breast cancer. Such studies have generated conflicting experimental and clinical reports of the role of infection in breast cancer. This review evaluates the current evidence for a productive oncogenic viral infection in human breast cancer, with a focus on the integration of sensitive and specific next generation sequencing technologies with pathogen discovery. Collectively, the majority of the recent literature using the more powerful next generation sequencing technologies fail to support an oncogenic viral infection being involved in disease causality in breast cancer. In balance, the weight of the current experimental evidence supports the conclusion that viral infection is unlikely to play a significant role in the aetiology of breast cancer.

1. Introduction

Breast cancer is the most common cancer to afflict women and accounts for approximately one quarter of all female cancers [1]. However, most breast cancers are sporadic and efforts to identify a unifying genetic or epigenetic cause can only explain a small proportion of disease. For instance, genetic variants which predispose to disease are present in approximately 30% of cases [2]. Intriguingly, in Queensland, Australia, the incidence of breast cancer increased from 80/100 000 in 1983 to 117/100 000 in 2002; equivalent to a 45% increase in incidence [3]. Similarly, in the United States of America, there was a 40% increase in the incidence over the 25 years to 2002 [4]. This increase in incidence may be linked to environmental factors that contribute to breast cancer development as exemplified by the increased incidence of breast cancer in Japanese women who migrated to the USA [5,6]. There are also accounts of 'cancer clusters' where high incidences of breast cancer are reported in work sites and amongst spouses [7,8]. Some of the increase in disease may be however linked to factors such as increased incidence of obesity [9]. Nonetheless, these observations have fuelled interest in a potential infectious aetiology for breast cancer.

Globally, it is estimated that 16% of all human cancers have an infectious origin [10]. Oncogenesis can be induced i) directly by viral genes, such as high-risk Human Papilloma Virus in cervical and mucosal head and neck cancer, ii) by viruses which reduce host immunity such as human immunodeficiency virus, and iii) by viruses which induce oncogenesis via chronic inflammation such as hepatitis B and C. Indeed, in 1936, it was observed that a transmissible form of mammary tumours in the mouse was caused by an extrachromosomal factor transmitted in breast milk [11], later identified as 'Mouse Mammary Tumour Virus (MMTV)' [12]. In all of these instances, tumours were associated with a high viral load which made virus discovery and analysis relatively simple.

Many different infectious agents have been investigated as potential carcinogenic agents in breast cancer, including Human Papilloma Viruses (HPV), MMTV and Epstein-Barr virus (EBV) [13]. However, significant controversy exists in the literature as to the possible role of infection with viruses or other pathogens in human breast cancer. This controversy has been fuelled by the ultralow abundance of viral DNA within the tissue. Furthermore, published

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reports which investigate the presence of virus in breast tissue vary vastly with respect to the nature of pre-analytical phase (e.g. sample type, nature of storage, sample preparation, laboratory practices) and analytical aspects (e.g. detection method, PCR or probe design, use of controls). This raises the fundamental issue of the most appropriate molecular techniques with which to detect the viruses. The debate which surrounds the putative association of viruses with breast cancer has become more polarised with the now standard use of next generation sequencing technologies.

This review will examine the evidence for viral infection as a causative agent in breast cancer and will reference both standard molecular biology techniques in addition to next generation sequencing data. Whilst breast cancer treatment has evolved significantly over the last 30 years, the identification of markers, events or indeed infection associated with disease could be exploited to develop new treatments or induce cures in patients. For this reason, it is important that a unifying rationale toward the analysis and design of pathogen-disease studies; in particular next-generation sequencing data, is used to guide sensible diagnostic and therapeutic interventions in the future.

2. Guidelines with which to critically review evidence

The original discovery in the 19th century that disease could be caused by microbes led to the development of Koch's Postulates; criteria which sought to establish a causative association between a microbe and disease. These principles were replaced by the Bradford Hill criteria, which recognise more contemporary notions in disease pathogenesis such as obligate carriers and viral infection [14]. These criteria for causality have been further refined to take into account 'molecular evidence' [15]. To truly prove disease causation by an infectious agent, these criteria must be considered. In particular, a putative pathogen genome should be detected in most disease cases, not be detected in non-diseased tissue, and the molecular evidence should be reproducible [15,16]. Thus, the first step in demonstrating causality between an infectious particle and cancer is to reproducibly detect the pathogen in the diseased tissue.

Using techniques such as *in situ* hybridisation and nested PCR to detect virus prevalence in breast cancer has produced highly variable results. For instance, high-risk oncogenic HPV prevalence has been reported to range from 0 to 86% depending on the molecular biology techniques employed and population analysed [13]. Furthermore, some of the data in the literature is lacking in experimental rigour; with regard to not using non-malignant controls or matched tissue controls, or omitting nucleic acid quality control and experimental positive and negative controls. Typically, earlier published reports used either PCR based amplification (often nested PCR) or *in situ* PCR to detect very low levels of virus DNA in breast cancer. However, few studies have generated evidence of active transcription of viral genes in breast cancer [17,18]. Viral oncogenes, such as HPV E6 and E7 are detected at high abundance and fairly ubiquitously in other virally mediated cancers [19,20], with the notable exception of 'hit and run' oncogenesis observed with bovine papilloma virus [21]. Recent advances in technology now allow for the detection of pathogens in next-generation sequencing data for both transcriptomic and genomic signatures of all known human viral pathogens. These next-generation sequencing efforts consistently failed to detect viral genomic material in breast cancer, thereby refuting a viral aetiology. However, regardless of the analysis technology or methods being employed, it is imperative that the criteria for disease causation are carefully considered in order to avoid exaggerating association without adequately proving causation.

3. Detection of viruses in breast cancer 'pre' next generation sequencing

We reviewed the literature which investigated the prevalence of four viruses – Human Papilloma Virus, Epstein-Barr Virus, Mouse Mammary Tumour and Mouse Mammary Tumour–Like Viruses and Bovine Leukaemia Virus in breast tissues using 'pre' next generations sequencing technologies. This data highlighted the disparity of prior reports which investigated the presence of viral genomic material in breast tissue. Table 1 summarises the literature, with published reports being deemed as supportive of a viral aetiology, inconclusive or refuting a viral aetiology. Supplemental data includes a more detailed review of all reports included in Table 1. For each manuscript, a judgement whether the data is inconclusive, supports or refutes a viral aetiology was made. The judgement was made on the basis of the molecular criteria for causality described above. For instance, many studies failed to examine normal tissue from non-diseased/benign breast tissue or adjacent normal tissue. These studies are inconclusive, as they do not determine the presence of pathogen in normal breast. Furthermore, studies which showed a virus prevalence in normal tissue which approximated the level in malignant tissue were also deemed inconclusive, as were studies which showed a prevalence of virus at 1 or 2% in breast cancer. Some studies were strongly supportive of a role for viruses in breast cancer, with viral DNA detected only in malignant tissue. Some studies failed to detect viral DNA in any tissue, or failed to detect it at increased prevalence in malignant breast. Some studies utilised antibodies to detect viral gene products by immunochemical methods. However, antibody based methods may not be sensitive enough to detect low level viral infection, and molecular based methods are considered more sensitive and specific.

Collectively, the literature demonstrates that in accordance with the population studied and the experimental methodologies employed, the detection of viruses in breast cancer is highly inconsistent. If one is to apply the guidelines with which to critically review evidence for an infection causing disease, many of these studies fail to provide sufficient evidence. Moreover, the volume of disparate data describing the prevalence of viral infection in breast cancer is not precedented in the literature for other truly virally mediated malignancies (such as other HPV-induced malignancies).

3.1. Human Papilloma Virus (HPV)

Hundreds of Human Papilloma Virus (HPV) subtypes have been described. The so called 'low-risk' HPV subtypes are the aetiological agent for cutaneous warts and anogenital warts [22], whilst 'high-risk' HPV (e.g. subtypes 16, 18, 11, 33) are an aetiological agent in uterine cervical cancer, anogenital carcinomas [23–25] and head and neck cancers [26]. High-risk HPV produces oncogenic proteins E6 (which promotes the degradation of p53) and E7, which bind to the Retinoblastoma protein (Rb) and disrupts Rb/E2F complexes [27]. Reports of HPV prevalence in breast cancer ranges from 0% to 86%, summarised in Suppemental Table 1.

3.2. Epstein-Barr Virus (EBV)

Epstein-Barr virus (EBV), also called human herpes virus 4 (HHV-4) is one of eight known viruses in the herpes family, and is one of the most common viruses in humans. It is the aetiological agent for infectious mononucleosis, and it is estimated that over 80% percent of 18 year olds show serological evidence of prior EBV infection [28,29]. EBV is associated with nasopharyngeal carcinoma, gastric cancer, endemic Burkitts lymphoma and a subset of