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Microbes and cancer: Coming a full circle

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Abstract

Microbes, particularly viruses, have had a chequered history in cancer research. Considered in the 1960s and 1970s as the main cause of cancers, a decade later microbes were set aside as inconsequential to the field. However, with confirmed links established between microbial infections and certain cancers in the last several years – *e.g.* human papillomavirus infection and cervical cancer, and *Helicobacter pylori* infection and gastric cancer – the field has slowly started to take notice of microbes again. Today, we know that about 15% of all cancer cases reported worldwide is due to microbes. Moreover, latest research findings suggest that viruses can be engineered to selectively target and kill cancer cells. A healthy microbiome in the human gut is also thought to aid responses to certain cancer therapies. Therefore, microbes appear to be back in the game now. Here we present a short perspective of the 'full circle' that microbes have come and their relevance to cancer research.

Keywords: Cancer-causing microbes, cancer-causing viruses, H. pylori and gastric cancer, virus-therapy for cancer.

Today we know that cancer is very much a genetic disease. The origins of cancer are embedded deep within the genetic makeup of cells – within the DNA – and are the result of certain "perfect combinations" of genetic alterations that affect our genomes (DNA) over time (the *perfect storm* (Zhu *et al*., 2016)). These alterations, or *damages* to the DNA as one may call them, can be caused by both *endogenous* as well as *exogenous* factors. For example, our cells constantly produce free-radicals called reactive oxygen species (ROS) as by-products of everyday metabolic or energy-producing activity; these ROS molecules are chemically reactive and can cleave our DNA, resulting in single- or double-strand DNA breaks (SSBs and DSBs). Similarly, (constant) exposure to UV radiation or cigarette smoke can alter the nucleotide sequence or result in an increase in the number of SSBs and DSBs. It is estimated that the DNA in our cells is exposed to 10,000 such onslaughts on average every single day. Our cells have evolved to protect themselves against these onslaughts, in the form of sophisticated *DNA-damage response* (*DDR*) mechanisms to respond and repair the damages almost instantaneously (Liu *et al.*, 2014). However, if the rate of these DNA damages is much higher than the DDR can cope with (*e.g.* DNA damage from cigarette smoke) or if the damage hits a gene that is itself responsible for the execution of the DDR, then a large number of genetic alterations can accumulate. Over time, some of these alterations that hit the *right combination* of genes (referred to as *oncogenes* and *tumour suppressor* genes) can cause the cells to go haywire – proliferate rapidly, not succumb to cell-cycle arrest and cell death despite carrying a high burden of DNA damage, and hijack other intra- and extra-cellular processes (*e.g.* metabolic) to aid them in these activities (*the hallmarks of cancer* (Hanahan & Weinberg 2000; Hanahan & Weinberg 2011) – thereby resulting in malignant transformation of these cells.

Although as early as Theodor Boveri (1862 – 1915), scientists (willingly or reluctantly) believed that cancer is a disease arising from cellular processes, much of what we know today about the exact mechanisms that underlie cancer initiation and progression were discovered only over the last two to three decades. In fact much of this understanding has come by only over the last 15 years, since the introduction of genome-sequencing and large-scale molecular-biology and bioinformatics techniques. However, up to the 1960s and to an extent the 1970s, failure to understand how cancer develops had made scientists speculate that cancer was perhaps caused by *microbes* – particularly *viruses* – that attack our cells and cause the cells to turn malignant. So much so that proponents of this theory began frantically hunting for these *cancer-causing viruses* (Mukherjee 2010). This meant that cancer could be infectious and spread from one person to another just like any other infectious disease: "Cancer may be infectious", the *Life* magazine had asserted in 1962 (Mukherjee 2010). However, today we know that this is true for only a very small fraction of cancers (about 15% of all cancer cases worldwide), and that vaccines have been developed against most kinds of these microbe-caused cancers.

Although the idea of microbes causing cancer looks pretty much an 'open-and-shut case' now, the quest for these microbes during the 1960s and 1970s has helped shape our understanding of the disease over time. The grandfather of the 'oncogenic virus' theory was Peyton Rous, who in 1910 at the Rockefeller, was able to inject cells from spindle-cell

sarcoma growing in one hen into another hen and successfully transmit the cancer. The cancerous cells in the recipient hen "grew rapidly, infiltrated organs and remained true to its cancer type" (Mukherjee, 2010). Rous didn't stop there. Rous filtered the cells through finer and finer cellular sieves expecting the cancer to stop transmitting; however, what Rous found was that the cancer continued to transmit and propagate. This meant that some *tiny* particle within the cancer cells was responsible for carrying the cancer, and the only explanation at that time was this particle was a virus. This virus came to be known as Rous sarcoma virus or RSV.

In 1935, Richard Schope, a colleague of Rous, reported a papillomavirus that was capable of causing cancers in rabbits. However, before that, in the late 1920s, a gynaecologist named Papanicolaou, who while working with cells from the cervix, noticed some "aberrant and bizarre" cells which looked very different from normal cervix cells. Soon it became apparent that these aberrant cells were in fact normal cells that had turned malignant (cancerous). Papanicolaou continued his work on these cells, and by the 1950s, he had developed a test using cell smears from the cervix to enable early detection of cervical cancer. This test – called Pap smear – pushed the diagnostic clock backward – from incurable cancers to early-detectable and curable malignancies (Mukherjee 2010). Eventually, in the 1980s a clear link was established between cervical cancer and the human papillomavirus (HPV): More than 99% of cervical cancer cases are caused by HPV infection. Since then more than 100 types of HPV have been discovered. Most HPV types spread through sexual or blood contact, however some types of HPV can also spread through oral sex. Vaccines against HPV infection are now available, and these are part of routine vaccinations in several countries.

Figure 1. Burkitt's lymphoma affecting children. (a) Seven-year old Nigerian boy with ulcerated tumour. (b) Tumour showing disruption of teeth and partial obstruction of airway. Source: Wikipedia, available under CC BY-SA 2.5 licence. Credit: Mike Blyth - Own work.

During the 1960s and 1970s, an Irish scientist Denis Burkitt, found an aggressive form of lymphoma, which is a cancer of the lymphatic system, affecting children in Africa (**Figure 1**). This lymphoma, which later came to be known as Burkitt's lymphoma, is a kind of Non-Hodgkin's lymphoma and affects the B cell lymphocytes. [Lymphomas are typically divided into Hodgkin's and Non-Hodgkin's lymphomas – HLs and NHLs – with the difference between the two being largely historical and the former first identified and described by the British physician Thomas Hodgkin. NHLs are usually more diverse and can affect lymph nodes across the body]. Much later, in the 1990s and 2000s, a link was established between Burkitt's lymphoma and the infection by a virus called Epstein-Barr virus (EBV). EBV lives unnoticed in as many as 95% of lymphoma

patients, and the viral products released by EBV contribute to both creating as well as maintenance of the cancer. EBV has now been associated with a few other kinds of HLs and NHLs as well.

The human immunodeficiency virus (HIV), the virus that causes acquired immune deficiency syndrome (AIDS), doesn't appear to cause cancers directly. However, due to a weakened immune system, the immune cells that aid in countering and killing newly formed cancer cells may not be able to do so as efficiently in AIDS patients. HIV infection has been linked to a higher risk of developing Kaposi sarcoma, cervical cancer, and certain kinds of NHLs.

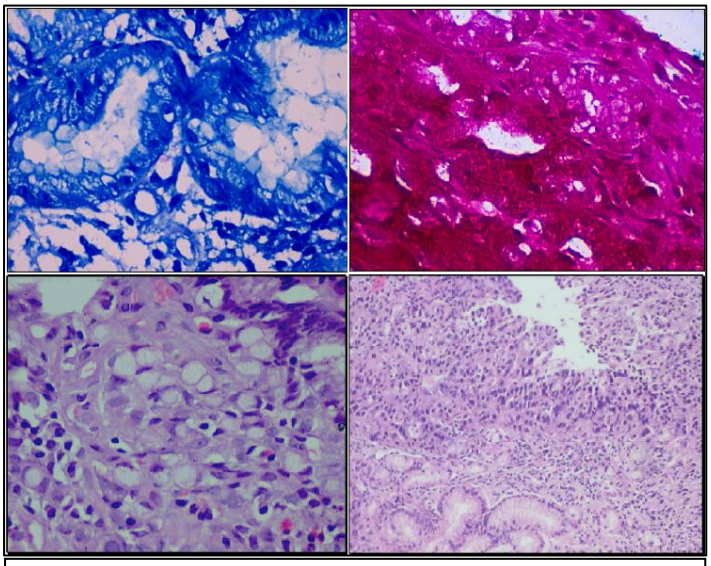

Figure 2. Carcinoma and *H. pylori* infection. Top panel: Staining of *H. pylori* in gastric biopsies. Bottom panel: Signet-ring cell and poorly differentiated gastric carcinoma. Image reused from Sulakshana *et al.*, (2015), with permission from the publishers.

A decade after Burkitt's discovery, in 1984, Barry Marshall, a junior resident at the Royal Perth Hospital, was working on another deadly microbe, this time a bacteria, called *Helicobacter pylori*. He found that the bacillus *H. pylori* was highly active in patients diagnosed with gastritis and ulcerating craters in their stomachs. Many of these cases of chronic gastric inflammation and ulcers gave way to gastric cancer. This raised the possibility that *H. pylori* infection and gastric inflammation increased the risk of developing gastric cancer.

A recent study in India demonstrated presence of *H. pylori* in gastric biopsies using special stains (**Figure 2** Top) and revealed that the bacilli were seen in 58 – 72% of gastric cancers including as rare as gastric lymphomas, but the majority (96%) of the cases were adenocarcinomas (**Figure 2** Bottom) (Sulakshana *et al.*, 2015). The number and density of the bacilli also correlated with the differentiation state of gastric carcinoma.

Today, we know that about 15% of all cancers worldwide are caused by microbes, in particular viruses. In addition to those highlighted above, hepatits B virus, human herpes virus-8, lymphotrophic virus type-1, and hepatitis C virus are capable of causing or contributing to cancers. Likewise, *H. pylori* has been classified as a class-I carcinogen by the International Agency for Research on Cancer due to its strong causal association with gastric cancers. The Center for Disease Control and Prevention (CDC) estimates that approximately two-thirds of the world's population harbors the bacterium, with infection rates much higher in developing countries than in developed nations.

Most of the cancer-associated viruses are *retroviruses*, which enter the nuclei of host cells, and insert viral DNA into the genome of cells. This new DNA material either carries a viral

oncogene (*v-onc*) or is inserted close to a proto-oncogene on the host genome causing overexpression of the host oncogene, and thereby resulting in oncogenic transformation of host cells. An alternative but rarer possibility for host cells to turn cancerous is from the defense mechanisms that are unleashed by host cells to remove the viral DNA: Host cells unleash a class of enzymes called APOBECs to chop out the viral DNA; but, occasionally these enzymes turn haywire and induce "random chops" in the host DNA (a double-edged sword), thereby causing insertions and deletions in the genome, and increasing the risk of oncogenic transformation. Recently, a study led by Michel Nussenzweigh at the Rockefeller found that an enzyme called activation-induced cytidine deaminase (AID) that helps create antibodies against diseases such as malaria can cause DNA damage that can lead to Burkitt's lymphoma (Robbiani *et al.*, 2015).

Back in the game?

Today, more than 200 different types of cancers potentially affecting 60 different organs have been identified. Given their growing number and heterogeneity, understanding the genetic basis of these cancers to develop effective therapies against them has become a crucial need. This has generated a flurry of efforts to develop *personalised* or *targeted* therapies against these cancers.

Viruses have had a chequered history in this quest for understanding cancer. Viruses were once thought to be the primary cause of cancers, but as time went by, ignored as inconsequential for the disease. With the discovery of new viruses and the infections these cause, new links have started to emerge between viral infections and some cancers. Importantly, with recent epidemics arising from Zika and the different types of H*N# influenza viruses, it may be only a matter of time until new links between these infections and some kinds of cancers begin to emerge. The development of antibiotic and antiviral drugs and vaccinations against cancer-causing microbes is achievable, as has been in the case of cervical (HPV) and gastric (*H. pylori*) cancers. Therefore, these microbe-caused cancers could very well be preventable or detectable early and cured. Recently, research has also indicated that viruses can themselves be used as weapons against cancers – by engineering viruses to provoke the host's immune response against cancer cells (Ledford 2015; Andtbacka 2015). In addition, there are some recent indications that beneficial bacteria in the human gut aid the immune system, and these bacteria may help to augment existing cancer therapies by slowing down tumour growth (Ornes, 2015). Therefore, it looks like microbes are back in the game now. What remains to be seen is how well our understanding of the link between microbes and cancer can be used to prevent, diagnose, and treat cancers.

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