Rationalisation of cancer therapy: modelling the physical and immunological tumour microenvironment

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Kok-Ho's interest lies in infectious diseases and oncology; in particular, the use of microbial therapeutics in cancer treatment. Kok-Ho's primary motivation in medicine is to find a novel therapy for cancers with poor prognosis.

The tumour microenvironment has emerged as an important field in carcinogenesis. For most of the 20th century, cancer therapies have focused predominantly on tumour cells. Despite our best efforts, these therapies remain ineffective against cancers with poor prognosis such as ovarian and pancreatic cancer. Studies have shown that the tumour microenvironment consists of a variety of epithelial and stromal cells which interact with one another and influence the outcome of treatment. By considering these interactions within a microenvironmental model of carcinogenesis, it may be possible to optimise cancer treatment strategies using not only conventional methods such as chemotherapy and radiotherapy, but emerging methods such as gene therapy and immunotherapy. This article will attempt to briefly illustrate the potential of translating microenvironmental characteristics into clinical practice by using a specific model of carcinogenesis.

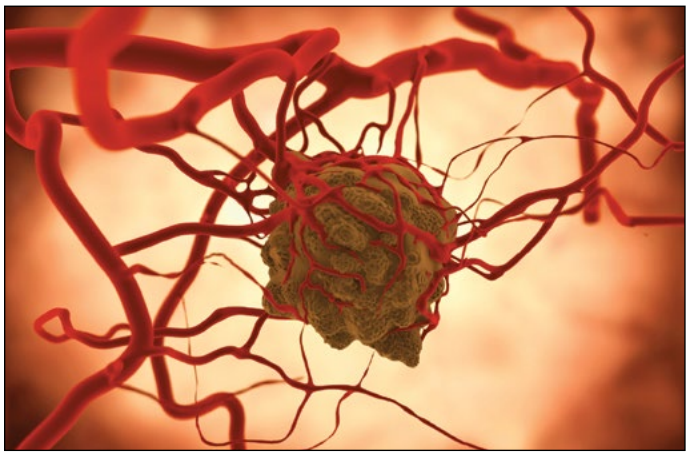
Introduction

In the 20th century, the somatic mutation theory has dominated our view of carcinogenesis. Cancer was viewed as a cellular phenomenon where genetic abnormalities result in aberrant cells that proliferate uncontrollably. [1,2] The theory, however, does not explain why certain cancers with known mutations (e.g. BRCA1/2 in breast cancer) only arise in a subset of patients; suggesting a role for non-genetic factors. [3] Thus, the tumour microenvironment is increasingly recognised as an important determinant of cancer progression.

In this environment, tumour cells co-exist with stromal cells such as fibroblasts and immune cells. Immune cells were first implicated in carcinogenesis by Virchow in the 19th century when he observed that leucocytes infiltrate into neoplasms and these sites of infiltration often correlated with chronic inflammation. [4] This inflammation is associated with autoimmune diseases (e.g. inflammatory bowel disease in colorectal cancer) and infections (e.g. *Helicobacter pylori* in gastric cancer) and generally involves tumour-promoting immune cells such as regulatory T-cells (Tregs), immature dendritic cells (DCs) and M2-polarized tumour-associated macrophages (TAMs). [5] Conversely, Fehleisen and Bruns found that acute inflammation had a tumour-suppressive effect. This was based on experiments whereby acute infections (e.g. erysipelas) of cancer wounds often resulted in tumour regression; [6,7] mediated in part by immune cells such as natural killer (NK) cells, CD8+ T cells (CTLs) and M1-polarized macrophages. [5] These two groups of immune cells differ in their cytokine profile. For example, M1 macrophages tend to produce pro-inflammatory cytokines such as interleukin-6 (IL-6), IL-12 and IL-23 which impede tumour progression while M2 macrophages produce anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 which promote tumour progression. [8]

Tumour-associated fibroblasts are also associated with carcinogenesis. They originate predominantly from the mesenchyme and can produce matrix metalloproteinases (MMPs) which degrade the extracellular matrix (ECM) and enable invasion of cancer cells. [9] Furthermore, fibroblasts are a potent source of growth factors such as vascular endothelial growth factor-A (VEGFA) which promote angiogenesis. [9]

In tumourigenesis, the vasculature can also be leaky and malformed; resulting in the formation of hypoxic and acidic regions due to a lack



of oxygen perfusion and a subsequent switch to anaerobic respiration. [10] Dysfunctional lymphatic vessels also significantly increase the interstitial fluid pressure within the ECM, thus impeding the entry of chemotherapeutic drugs by transcapillary flow and convection. [11] As these drugs are also oxygen or pH sensitive (e.g. doxorubicin), the efficacy of chemotherapy is severely limited.

To understand the role of the tumour microenvironment in carcinogenesis, it is essential to visualise its features in the context of a model. It is important to note, however, that this article is not comprehensive; rather it is meant to highlight points of interest for further reading.

Developing a microenvironmental model of carcinogenesis

A multistep model for carcinogenesis was proposed by Fearon and Vogelstein in 1990. Based on observations documented in human colorectal cancer, they linked changes in tumour morphology to specific mutations in oncogenes and tumour suppressor genes. [12] They proposed that accumulation of mutations, rather than sequence, was seen as the most important determinant of tumour progression. [12] In 2000, Hanahan and Weinberg proposed that six acquired traits are required for the formation of an invasive cancer. These include: self sufficiency of growth signals, insensitivity to anti-growth signals, resistance to apoptosis, unlimited replication, sustained angiogenesis and invasiveness. [13]

Subsequently, studies have shown that the microenvironment can affect the development of cancer cells. [14] Fuelled by the tissue organisation field theory (TOFT), it was believed that tissue homeostasis is controlled by stroma-epithelium interactions that impose environmental barriers to tumour progression. [15] During tumourigenesis, such barriers go awry and affect the type and sequence of phenotypic strategies adopted by cancer cells (that is, observable characteristics manifested by cancer cells to override barriers). [16]

Finally, in 2007, Gatenby and Gilles proposed a sequential microenvironmental model. [16] In this model, the initiating event is insensitivity to anti-growth signals mediated by contact inhibition (arrest of growth by cell contact). [17] Tumour cells escaping from the basement membrane (BM) survive by up-regulating growth factors and/or their receptors to acquire self-sufficiency; illustrating the ability

of tumour cells to contribute to signals in the microenvironment [18,19] As proliferation occurs, tumour cells are constantly constrained by senescence (through cellular ageing by telomere shortening) and therefore require increased telomerase activity to avoid apoptosis. [20] Conditions become more hypoxic in distal regions of the tumour and distal cells adapt by switching to glycolysis and increasing glucose uptake. [21] This altered metabolism results in a low extracellular pH which is toxic to tumour cells. To counter this situation, cells may acquire p53 mutations or increase Na^+/H^+ exchanger activity. [16] Alternatively, transcription factor hypoxia-inducible factor 1 (HIF-1) may be up-regulated in response to hypoxia, resulting in a gene expression profile (for example VEGF and glycolytic factors) which overcomes both hypoxia and acidosis. [22]

Despite these strategies, the poorly formed vasculature is usually unable to match the tumour's demand and nutrients need to be supplied by angiogenesis. [23] Increasingly unfavourable conditions eventually promote tumour cells to be invasive by favouring motile cells which can spread to adjacent normal tissue.

The abbreviated development of a microenvironmental model is summarised in Figure 1.

From the Gatenby and Gilles model, several principles can be extrapolated. [16] First, carcinogenesis can be viewed as a sequence of phenotypic strategies selected by conditions in the microenvironment. Second, different phenotypic strategies can be utilised by the tumour as long as they confer the same selective advantage towards overcoming unfavourable environmental conditions. Third, phenotypic strategies at a particular stage of tumourigenesis may impede or promote progression through subsequent environmental barriers.

Targeting the tumour microenvironment

If carcinogenesis progresses in a sequential manner, appropriate treatment can be initiated at specific stages of progression. Withstanding the variability of different cancers, the Gatenby and Gilles model offers good insights by proposing one of many possible progression sequences. The challenge to this phenotype-specific approach lies in the ability to model the different cancer microenvironments and the prompt detection of cancerous changes; many cancers (for example, gastric cancer) are not detected at the early stages of disease. [24] Recent advances in molecular and mechanical characterisation methods have been encouraging. Using a proteomics approach, Ryu et al. have identified two groups of proteins which are over-expressed (for example, transgelin and prohibition) and under-expressed (for example, desmin and serotransferrin) in gastric cancer while Vakoc et al. have developed an advanced form of three-dimensional microscopy known as optical frequency domain imaging (OFDI) which is capable of investigating huge tissue volumes and dynamic tumour changes over long periods. [25,26]

Targeting inflammation as a sequential entity

The sequential transition of acute to chronic inflammation may be involved in tumour progression. Cancer and infections, autoimmunity and graft rejection share biphasic patterns characterised by waxing and waning of immune responses. [27] These patterns may be attributed to a common immunological constant of rejection. Mantovani et al. hypothesised that inflammation can be divided into two tiers. The first tier is thought to be a baseline level of inflammation mediated by activation of interferon-stimulated genes (ISGs). [28] This was based on experimental evidence showing a convergence of ISG expression in cancer and chronic states such as persistent hepatitis C virus (HCV) infections and long-term transplant rejections treated with immunosuppressants. [29] Conversely, the second tier is a cytotoxic-mediated inflammation provided by CTLs and NK cells. This is more commonly seen in tumour regression, acute exacerbations of inflammatory bowel diseases and acute hepatitis C-mediated liver cirrhosis. [27]

From Mantovani's hypothesis, two possible directions can be pursued: controlling the chronic baseline level of inflammation or promoting the cytotoxic effector functions of immune cells. Controlling chronic inflammation may be achieved by treating the underlying trigger such as infections (for example, HCV in hepatitis C and *Helicobacter pylori* in gastric carcinoma) or prevention using anti-inflammatory drugs. [30] The use of non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with regression of established tumours or inhibition of pre-malignant lesions in cancers such as gastric and colorectal cancers. [31]

Targeting immune cells: enhancing cytotoxicity via innate immunity

Promoting the cytotoxic function of immune cells would require overcoming tumour-induced immunosuppressive networks (for example, Tregs and suppressive cytokines). Vaccination strategies involving tumour antigens and *ex vivo* activated T cells have been used to stimulate localised anti-tumour responses but results are

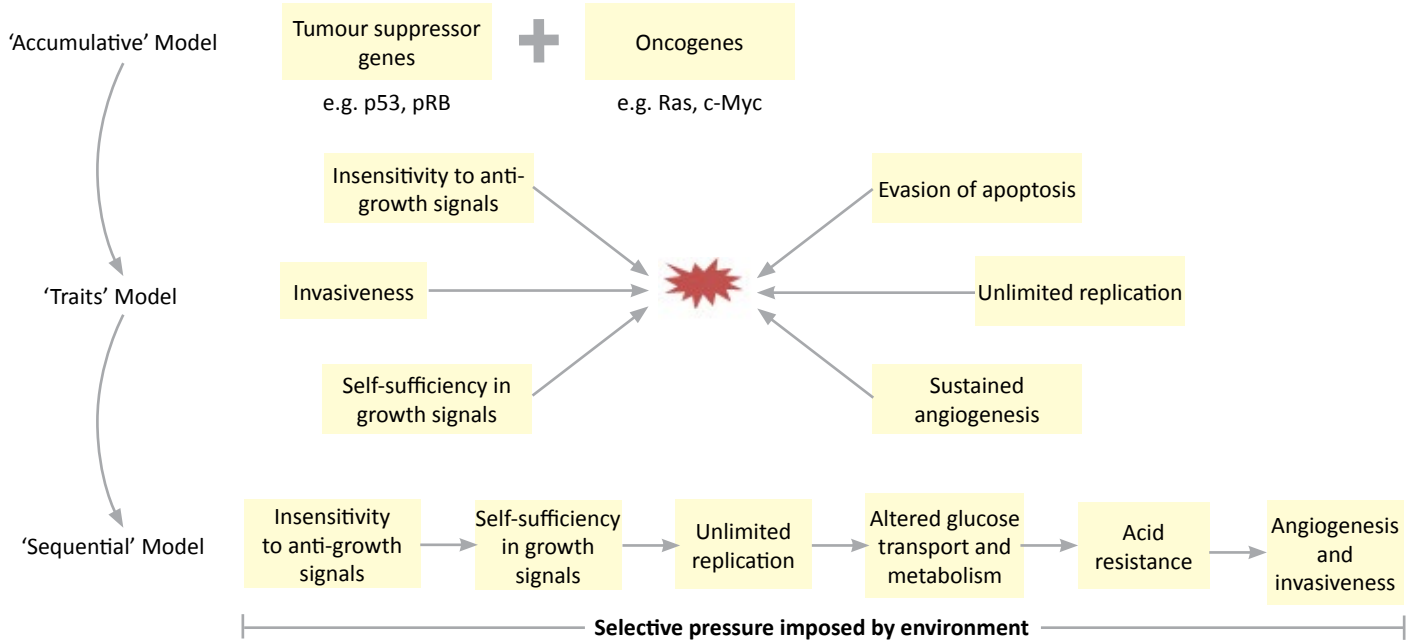


Figure 1. *Developing a microenvironmental model. Early researchers focused on the accumulation of TSGs (for example, p53 and pRB) and oncogenes (for example, Ras and c-Myc) as the driver for carcinogenesis. This was later replaced by an emphasis on understanding the different traits required for tumour survival. Growing recognition of the tumour microenvironment will necessitate a sequential model driven by selective pressures imposed by environmental barriers.*

disappointing because the role of innate immunity in shaping adaptive immune response has been neglected. [32] Effective CTL response requires co-stimulatory molecules. These molecules are up-regulated in response to pathogen-associated molecular patterns (PAMPs, such as lipopolysaccharides) interacting with toll-like receptors present in innate immune cells (such as DCs and macrophages). [33] It has been shown that interaction of TLR9 with CpG oligodeoxynucleotides results in high levels of anti-tumour interferon alpha (IFN-α) production while TLR7 agonists have been used to treat basal cell carcinoma via an IFN-γ mediated mechanism. [34,35] However, as some TLRs (namely TLR2 and TLR4) are also involved in chronic inflammation, TLR antagonists have been increasingly researched as potential anti-cancer agents. [36] A recent study also showed that a subset of DCs known as interferon-producing killer DCs can combine antigen-presenting and cytotoxic functions; suggesting that innate cells have an understated importance in tumour suppression. [37]

The importance of innate cells is further illustrated by NK cells. Natural Killer cells function as an important link between innate and adaptive immunity as they are involved in DC maturation which then facilitates priming of effective CTL responses. [38] Illustrating the third principle, tumours are shown to reduce major histocompatibility complex (MHC) Class I expression as a means of evading CTLs and this renders them sensitive to NK cell-mediated cytotoxicity [39]. Nonetheless, low NK cell count in tumours (due to lack of chemokines or proliferative cytokines) may explain why tumours continue to progress. Studies have shown that intratumoural injection of CpG oligodeoxynucleotides can improve NK cell infiltration while Treg depletion followed by IL-15/hydrocortisone induction significantly induced higher NK cell numbers. [40,41] Similarly, induced pluripotent stem cell (IPSC) derived NK cells have been shown have a positive effect on tumour regression in several cancers. [42,43] These observations suggest that NK cell-based immunotherapy can be effective.

Targeting single cytokines or cells involved in cytokine networks?

To date, only two cytokines have been approved for cancer treatment: IL-2 and IFN-α. [44] IL-2 was initially tested in the 1980s a means of treating advanced solid cancers. It is worth postulating that their efficacy probably lies in some cancers exhibiting limited phenotypic strategies; thereby explaining why few cytokines have been approved. [45] Although a favourable response was found in renal cell carcinoma patients, efficacy was limited at 30% while IL-2 was implicated in the potentially fatal capillary leak syndrome. [46] Furthermore, IL-2 used to expand CTL subsets was also found to increase Treg numbers; effectively down-regulating the initial cytotoxic response. [47] Inhibition of cytokines implicated in tumourigenesis (such as IL-6) by monoclonal antibodies has also been considered but this approach has been largely limited as IL-6 receptors are not tumour-specific. Tumours have complex cytokine networks; these cytokines work in tandem and cancers may exploit multiple cytokines to achieve progression. [48] Limiting one arm of this network may simply activate another; thus requiring us to consider cytokine therapy on an integrative level.

Targeting cells involved in cytokine networks may achieve the same effect as administering multiple cytokines. For example, two potential targets for TAMs include the interferon response factor 5 (IRF5) and the Src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1). Interferon response factor 5 is a transcription factor that is involved in M1 phenotype polarization (high levels of IL-12 and IL-23, low levels of IL-10). [49] The other target SHIP1 is a phosphatase that is also involved in M1 macrophage polarization and is a potent repressor of M2 polarization. [50] Experiments demonstrated that mice deficient in SHIP1 not only develop M2 phenotype macrophages but also have a higher incidence of tumour formation. [50] Both IRF5 and SHIP1 may potentially be targeted by gene silencing techniques (such as RNA interference) in the future. [51]

Targeting the physical environment: The example of HIF-1

Hypoxic strategies may underlie the importance of an integrated treatment approach in targeting multiple phenotypic strategies. Low oxygen content can result in tumour cells that adapt by inducing HIF-1 expression. This leads to reduction in oxygen consumption by the mitochondria as well as increased transport and anaerobic metabolism of glucose. [52] In response to HIF-1, vascular endothelial growth factor (VEGF) may also be up-regulated in the tumour microenvironment to improve oxygenation by angiogenesis. [53] Anti-VEGF agents such as bevacizumab were initially used to inhibit angiogenesis in colorectal cancer but researchers later realised that it may normalise tumour vasculature and paradoxically lead to higher oxygen levels and increased risk of metastasis. [52] Although oxygenation potentially improves radiotherapy and oxygen-dependent chemotherapeutic drugs like etoposide and mephalan, there may be a trade-off between improving short-term outcomes at the expense of tumour persistence. [54] It has been postulated that cancer stem cells (CSCs) may be found proximal to blood vessels. [55] CSCs are tumourigenic cancer cells capable of self-renewal and differentiation into all cancer cell types and are refractory to many forms of chemotherapy; promoting survival of these cells therefore predisposes to cancer regeneration. [56] Multiple phenotypic strategies can be targeted specifically to restrain tumour cells from exploiting alternate pathways. Where possible, targeting a pleiotropic transcription factor such as HIF-1 by inhibitors such as phenethyl isothiocyanate (by translation inhibition) may achieve the same outcome as targeting multiple strategies. [57] This is because HIF-1 trans-activates many genes involved in the tumour's phenotypic strategies (including VEGF, mitochondrial regulators and glycolytic enzymes). [22]

The third principle states that phenotypic strategies may have paradoxical effects on tumour progression. While HIF-1 leads to a switch to glycolysis and also helps overcome acidosis and ischaemia, other phenotypic strategies may increase the threshold for future barriers. [16] The initial phenotypic strategy of avoiding cell death after BM detachment permits distal cell proliferation but consequently, subsequent 'thresholds' of environmental barriers are increased as highlighted by higher degrees of growth signal insufficiency and hypoxia that impede distal tumour cells. [16]

These observations have implications for treatment. First, strategies that lower the threshold for subsequent barriers should be actively targeted since they may hasten the progression of malignancy. Second, strategies that increase the threshold may result in low to mid-grade malignancies that are amendable to our current conventional approach. For example, basal cell carcinoma is typically a low-grade malignancy that is highly responsive to radiotherapy and surgical excision. [58]. These observations may suggest why early-stage neoplasms tend to have a favourable prognosis, whereas late-stage malignancies probably exhibit features of threshold reducing strategies and are thus more aggressive.

Further considerations: Combination therapies and multiple microenvironmental 'niches'

The vast array of components in the tumour microenvironment not only highlights the importance of integrative treatment but also the need for combination therapies focusing on multiple treatment modalities. The latter may be relevant to highly resistant cancers that are likely to exhibit phenotypic strategies that reduce the threshold of environmental barriers. An example of combination therapy is the use of low-dosage cyclophosphamide with dendritic cell-based immunotherapy in mesothelioma. [59] While mesothelioma is generally resistant to most cytotoxic chemotherapeutic drugs such as cisplatin and docetaxel, low-dosage cyclophosphamide can potentiate the immune-stimulatory effects of DC vaccination via inhibition of Tregs. [60] Thus, combination therapies can essentially bypass the ability of cancers to circumvent some environmental barriers by increasing the threshold of other barriers.

Tumour progression is also a dynamic process and when considering the microenvironment in invasive cancers, this is in reality a combination

of different unique 'niches' composing of primary and metastatic sites. [61] This will inadvertently complicate the treatment process due to heterogeneity of different tumour environments but it is reasonable to assume that targeting the primary site will still be of important therapeutic value due to the role of the primary tumour in 'seeding' metastases and also possible parallel progression of primary tumours and metastases. [62]

Based on all three principles and the examples highlighted, rational treatment strategies may be designed (Figure 2).

Conclusion

In 2008, it is estimated that almost 7.6 million deaths were attributed to cancer. [63] Almost one in two patients treated for invasive cancer eventually succumb to the disease or the treatment. [64] As such, the design of current treatment strategies may be further improved to reduce mortality rates.

The medical practitioners of tomorrow will be given new tools in the fight against cancer. It is against this backdrop that devising rational treatment strategies becomes essential. The examples described suggest the importance of tumour characterisation and tailoring different approaches to these characteristics. Although this review only focused on a single model and specific examples, there can be more than one model of carcinogenesis for different cancers and many more treatment targets are under consideration.

The challenge to devising a good treatment strategy would be selecting the appropriate target(s) and considering the need for integrative/combination or conventional single-modality approaches. In particular, combining different treatment modalities may be crucial for persistent cancers and should be the focus of current research.

Conflict of interest

None declared.

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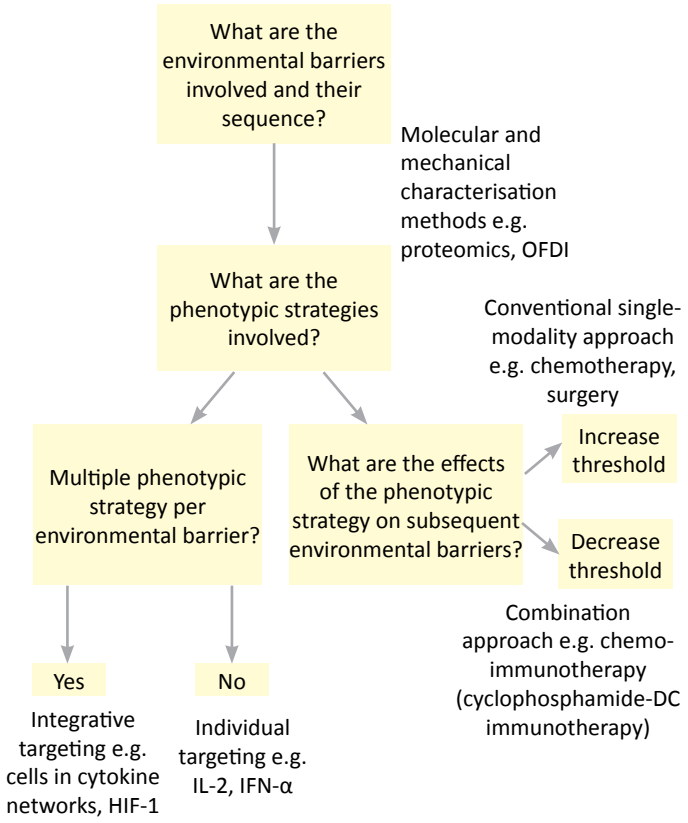

Figure 2. *Devising rational cancer treatment strategies Molecular and mechanical characterisation methods can be used to determine environmental barriers and their corresponding tumour phenotypic strategies. Integrative targeting (for example, cytokine networks) may be useful against multiple phenotypic strategies while individual targeting (for example, single cytokines) may favour cancers with limited strategies. The effect of phenotypic strategies on the level of threshold may also determine the type of treatment approach-single modality for increased threshold and combination for decreased thresholds.*

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