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Microcompetition and the origin of cancer

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Abstract

Abnormal gene expression is a common observation in cancer cells. Although genetic alterations via somatic mutations or DNA modifications are considered to be the cause of cancer, they do not explain the observed abnormal gene expression of many wild-type genes in cancer. Now, a new theory, called ''Microcompetition'', identifies a non-genetic-alteration event as the cause of the observed abnormal gene expression, and therefore, the cause of cancer and other chronic diseases.

Keywords: Microcompetition; Cancer; Foreign N-boxes; Limiting transcription factor; Cellular allocation

1. Introduction

Cancer is a complex, multi-step process. Although important advances have been made over the last 3 decades in understanding the genetic changes associated with cancer, the origin of the disease remains unknown. Now, a book by Hanan Polansky entitled ''Microcompetition with Foreign DNA and the Origin of Chronic Disease'' [1] provides a new perspective on the basic mechanism associated with the disruption underlying cancer.

The book introduces a new theory derived from recognition of patterns in reported observations. The observations represent isolated dots, which when connected generate a pattern in full view, namely a theory that identifies the disruption and the sequence of subsequent genetic, cellular, and clinical events associated with cancer.

Dubbed ''microcompetition'', the theory propounds that foreign DNA can compete with cellular DNA for cellular transcription factors resulting in abnormal gene expression and disease. The concept is not limited to cancer, but applies to an array of chronic diseases which show abnormal gene transcription. A common source of foreign DNA discussed in the book is infection by a latent virus whose genome can persist indefinitely in cells. Several latent viral genomes have been associated with human cancer and latent infection was also found in other chronic diseases.

The book derives numerous microcompetition-based predictions and documents observations from a large number of studies consistent with the derived predictions. The book explains some puzzling observations in cancer research where genetic changes are not apparent around the control regions of dysregulated genes, and provides a mechanism for the action of latent viruses where expression of a viral protein is not readily detected. In explaining the relationship between latent viral infection and disease, the book introduces a new protein-independent paradigm, which is both logically congruent and empirically consistent with observations reported in an extensive number of studies performed under variety of experimental conditions. This perspective reviews the main points of the microcompetition theory as it pertains to the origin of cancer.

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2. Limitations of the conventional paradigm in cancer

The prevalent view of the nature of cancer holds that it is a complex genetic process resulting from the progressive accumulation of mutations in specific cellular genes, such as proto-oncogenes or tumour-suppressor genes, leading to perturbations in processes involving signal transduction, cell cycle regulation, and/or apoptosis [2]. Genetic instability in tumours has been known for decades [3]. However, the role of genomic instability in causing and promoting tumour growth remains controversial [4,5]. Furthermore, although many studies report abnormal gene expression in cancer cells, often, no mutations or chemical modifications are observed around the locus of the dysregulated gene(s), suggesting that a genetic alteration is not the initiating event of cancer [1].

Polansky's book cites specific examples illustrating the discrepancy between aberrant gene regulation and DNA mutation or modification. For instance, the breast cancer-associated gene type 1 (*BRCA1*) has been reported to show decreased transcription in a majority of sporadic breast and ovarian tumours [6–8]. Similarly, the gene *Fas* (Fas, APO-1, CD95) was reported to have decreased transcription in several types of carcinomas [9–11]. In both cases, analysis of the respective genetic loci showed that the two possible causes, namely somatic mutation and promoter hypermethylation, were not involved. Hence, Polansky suggests that some other mechanism must be responsible for the observed decreased transcription of these genes (see below).

Another misconception in cancer pertains to the mechanism of cellular transformation by oncogenic viruses. Conventional thinking maintains that transforming viruses induce cell proliferation through the action of a viral-encoded protein. Accordingly, a latent viral infection is considered to be mostly harmless to the cell since little or no viral protein is made in infected cells. However, the book cites a number of instances of cancer associated with latent viral genomes.

3. The microcompetition theory

In contrast to the protein-dependent paradigm of latent infection in cancer, the book proposes that viral DNA itself may exert an effect on the cell in the absence of protein expression. Such a protein-independent effect could occur through competition between the viral and cellular DNA for a limiting transcription factor resulting in abnormal expression of cellular genes. To explain the aberrant transcription of genes in cancer and other chronic diseases, which cannot be explained by traditional methods, the author gives the following example. Consider the transcription factor GA binding protein or GABP (also called nuclear respiratory factor 2 (NRF-2)) [1]. This protein binds the promoter and enhancer of many cellular genes, including *BRCA1* [12], *Fas* [13] and retinoblastoma (*RB1*) [14]. Viral DNA can also bind GABP [1]. It has been found that many viral enhancers contain a core binding sequence that resembles the DNA box (N-box) to which GABP binds. The book cites a number of studies showing the binding of GABP to viral enhancers. Based on the use of viral enhancers in vectors and their proven effectiveness in transfection studies (e.g., cytomegalovirus immediate-early enhancer), it is suggested that viral enhancers may have a higher capacity than cellular DNA to compete for binding to transcription factors.

The GABP transcription factor does not function alone. It requires a family of coactivator proteins collectively termed p300/cbp, which interacts with GABP to form a transcriptional activation complex designated, GABP·p300/cbp. The coactivator proteins are available in limiting amounts, thereby rendering the transcription complex itself limiting in its availability. Studies are cited to show that competitive inhibitions of the p300/cbp coactivator proteins by other cellular or viral proteins lead to inhibition of transcriptional activation.

The microcompetition theory can be illustrated as shown in Fig. 1. In normal cells, the GABP·p300/cbp transcription complex binds to N-box sequences at the promoter region of a GABP-regulated cellular gene (e.g. *BRCA1*) and activates the expression of the gene. The author considers an infection of the cell by a persistent virus (designated "GABP virus") containing a GABP-binding site (viral N-box) in its enhancer, and inquires the effect of the infection on the *BRCA1* gene. Upon infection, the viral N-box would compete with the cellular N-box for binding to GABP·p300/cbp and sequester the transcription complex to the viral enhancer. Since GABP·p300/cbp is limiting, binding to the viral enhancer would decrease its availability to the *BRCA1* promoter, resulting in decreased *BRCA1* transcription. This is microcompetition, i.e., competition between a regulatory viral sequence and the promoter/enhancer of a cellular gene for a limiting transcription complex that results in altered expression of the cellular gene.

If the transcription complex transactivates the cellular gene, competition with the viral N-box would inhibit transcription. If the complex suppresses transcription, the competition would have the opposite effect. Further, an increase in the copy number of the viral regulatory sequence would amplify the effect of microcompetition between the foreign and cellular DNA.

The key point of the theory is that the competing DNA sequences do not bind each other, but compete for binding to a limiting transcription complex. In the example cited, the viral DNA and *BRCA1* gene do not bind each other, but compete for binding to the limiting GABP·p300/cbp transcription complex (Fig. 1). It is

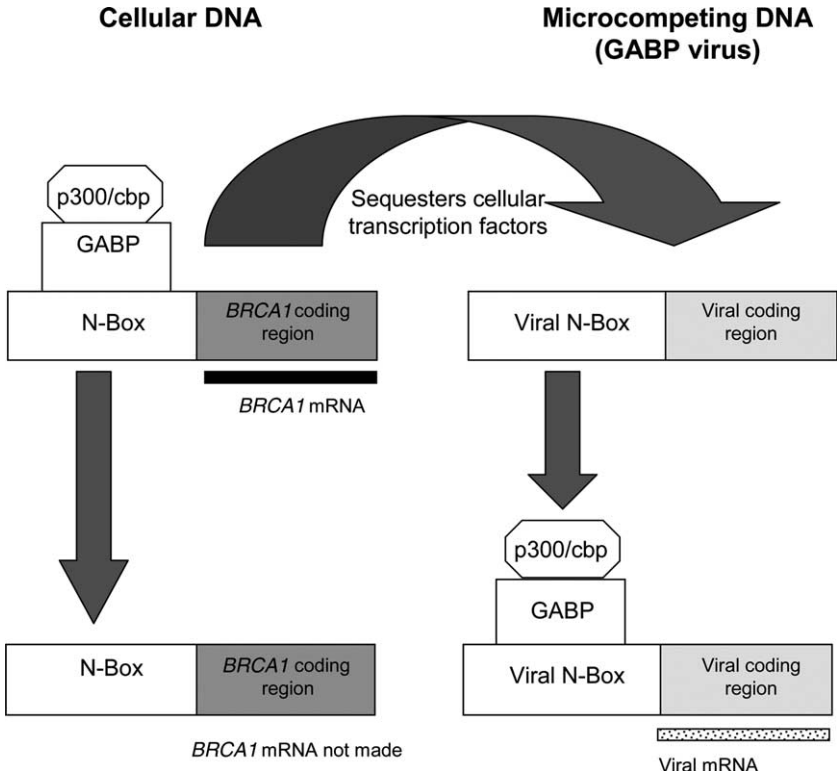

Fig. 1. Schematic diagram designed to illustrate microcompetition by foreign DNA. Shown on the left is cellular DNA containing a GA binding protein (GABP)-regulated gene (*BRCA1*) with N-box sequences in its promoter region. On the right is microcompeting DNA from a GABP virus with foreign N-box sequences in its enhancer. In normal cells (upper left), a complex of transcription factors (GABP plus p300/cbp) binds to the N-box at the *BRCA1* promoter and activates its expression, resulting in increased concentration of *BRCA1* mRNA. Upon infection of the cell by the GABP virus (upper right), the transcription complex bound at the promoter of the *BRCA1* gene is sequestered away by the N-box in the viral enhancer (lower right), resulting in decreased expression of the *BRCA1* gene (lower left) that is associated with cancer.

interesting that when explaining observations reported in the literature, biologists tend to rely on the traditional physicochemical philosophy which centres on binding/non-binding events, or physical contact between molecules. In contrast, microcompetition with foreign DNA, which in essence is a reallocation of a rare resource, seems to draw on economic rather than physicochemical principles.

Limiting transcription factors have been implicated in cell differentiation and development, but not in disease. The p300 protein is known to be involved in regulation of gene expression through movement from one DNA to another. In this case, foreign DNA distorts the allocation of p300 between genes. This model extends the allocation concept to chronic disease. Since chronic disease develops over a long period (several years), a disruption that affects genes slightly rather than dramatically is desired. The microcompetition model is consistent with such effect.

4. Evidence supporting the microcompetition theory

To test the microcompetition theory, Polansky presents logical predictions and compares the predictions with observations from studies published in the literature. Numerous examples are reported where experimental observations corroborate the predictions, thereby validating the theory. Some of these are discussed below.

One example deals with predictions and observations pertaining to the *RB1* tumour suppressor gene. Studies are cited to show that increased transcription of this gene and/or accumulation of an under-, or hypo-phosphorylated protein product correlates with cellular growth arrest and differentiation. Since the latter are inversely related to cell proliferation, increased transcription of Rb has an inverse effect on cell proliferation.

Rb is a GABP-regulated gene containing an N-box in its promoter. According to the microcompetition theory, an infection of a cell with a GABP-binding virus should lead to increased cell proliferation. This prediction is symbolically presented to show a series of quantitative events which predict the effect of foreign N-boxes on cell number. The quantitative events describe competition between the foreign DNA and Rb for the same limiting transactivation complex, which would result in decreased Rb transcription and increased cell proliferation.

To test this prediction, a set of transfection studies are described that used the same experimental design. These studies examined the effect of a test gene on cell function. In these studies, the test gene was inserted into a standard plasmid and transfected into indicator cells. Controls consisted of cells transfected with the standard plasmid without the test gene (designated "empty" vector) and non-transfected cells (designated "wild-type"). Polansky points out that in all the studies he reviewed, the authors compared the cells transfected with the test gene with those transfected with the "empty" vector or with the wild-type cells. None of the studies compared the cells transfected with "empty" vector with the wild-type cells. In contrast, Polansky closely examined the effects of the "empty" vectors.

The "empty" vectors consisted of some of the commonly used laboratory vectors, namely pZIP-neo, HSV-neo, pSV-neo and pcDNA3, which contain a viral promoter/enhancer. pZIP-neo and HSV-neo express the neomycin resistance gene under the control of the long terminal repeat (LTR) from the Moloney murine leukaemia virus [15] and Harvey sarcoma virus [16], respectively. pSV-neo expresses the *neo* gene under the control of the Simian Virus 40 (SV40) early promoter [17], and pcDNA3 vector carries the cytomegalovirus (CMV) promoter [18].

Polansky points out that the viral promoter/enhancer used in each of the above "empty" vectors contains a GABP binding site. Hence, the prediction was that the "empty" vector should have an effect on cell function, specifically, increase proliferation or decrease growth arrest/differentiation depending on the phenotype evaluated in the respective study. To test the prediction, Polansky carried out the unusual comparison of the cells transfected with the "empty" vector to the non-transfected (wild-type) cells. The result that he obtained was most unexpected by conventional thinking, but consistent with the microcompetition theory. In each case, Polansky found that transfection with the "empty" vector either decreased cell differentiation or increased cell proliferation depending upon the endpoint of the study. The result is unlikely to be an artifact of any given study since the same result was obtained under a variety of different experimental conditions using dissimilar methods. This is significant since the same result under dissimilar conditions is considered reliable, adding further confidence in the validity of the microcompetition theory.

In addition, other observations, not reported in the book, are consistent with the microcompetition concept. It is well known that subgenomic DNA fragments from herpes simplex virus type 2 (HSV-2), which overlap with the ribonucleotide reductase (RR) gene, can transform cells to the neoplastic phenotype [19,20]. The minimal transforming regions of HSV-2 DNA have been localised to fragments smaller than the size of the complete RR gene, indicating that an intact viral protein is not required to induce transformation [21,22].

In one series of molecular studies involving the minimal transforming region III (mtrIII) of HSV-2 DNA, constructs were prepared containing mtrIII DNA ligated to a heterologous viral promoter in an expression vector [23]. Such vectors expressed the N-terminal domain of the viral protein (RR large subunit) and conferred enhanced transformation compared with mtrIII DNA cloned in standard (promoterless) vector. However, when expression of the RR protein domain was abolished by the introduction of translation stop codons within the RR reading frame, the mutant DNA was still capable of transforming cells, albeit at a reduced level, comparable to that induced by wild-type DNA in the standard vector. These observations in essence indicated that mtrIII DNA could transform cells in the absence of viral protein expression. The authors of the report concluded that "The data are consistent with an independent role for mtrIII DNA, functioning possibly as a promoter-enhancer or a receptor for binding cellular regulatory factors" [23]. Subsequent to that report, N-box type sequences capable of binding to GABP have been detected in mtrIII DNA (Jariwalla and colleagues, data not shown). These observations are consistent with the microcompetition theory and provide additional support for its validity.

Polansky also makes predictions pertaining to cell migration in metastasis and describes observations from published studies that support the predictions. He also describes other disruptions that can lead to cancer, such as exogenous agents influencing GABP kinase phosphorylation or substances (e.g., nicotine) influencing oxidative stress. Dephosphorylation of GABP kinase or excessive oxidative stress could alter p300 allocation leading to increased cell proliferation and cancer. The author points out that although microcompetition with foreign DNA is only one disruption underlying cancer, the frequent detection of viral genomes in human tumours, often in an unexpressed latent state, would suggest that this disruption may be a widespread cause of cancer.

5. Conclusions

The microcompetition theory introduced in Polansky's book is based on three discoveries: (i) a new mechanism, allocation of limiting transcription resources among genes; (ii) a new disruption, the effect of foreign DNA on the cellular allocation of limiting transcription resources; (iii) a new set of logical steps, the sequence of events that lead from the introduction of foreign N-boxes into a cell to cancer. According to the microcompetition theory, the foreign N-boxes compete with cellular genes for a limiting transcription complex leading

to abnormal gene expression and disease. Observations from a large number of published studies support the predictions and validate the theory. In addition, the microcompetition theory provides a convincing molecular explanation for key observations reported in the cancer literature which are not adequately explained by traditional methods. Unlike prevalent explanations based on cellular genetic alteration or virus-mediated protein expression, the microcompetition theory introduces a *non*-genetic-alteration, viral-protein-*in*dependent event as the cause of cancer.

Conflict of interest statement

None declared.

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