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Review

Evasion of anti-growth signaling: A key step in tumorigenesis and potential target for treatment and prophylaxis by natural compounds

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ABSTRACT

The evasion of anti-growth signaling is an important characteristic of cancer cells. In order to continue to proliferate, cancer cells must somehow uncouple themselves from the many signals that exist to slow down cell growth. Here, we define the anti-growth signaling process, and review several important pathways involved in growth signaling: p53, phosphatase and tensin homolog (PTEN), retinoblastoma protein (Rb), Hippo, growth differentiation factor 15 (GDF15), AT-rich interactive domain 1A (ARID1A), Notch, insulin-like growth factor (IGF), and Krüppel-like factor 5 (KLF5) pathways. Aberrations in these processes in cancer cells involve mutations and thus the suppression of genes that prevent growth, as well as mutation and activation of genes involved in driving cell growth. Using these pathways as examples, we prioritize molecular targets that might be leveraged to promote anti-growth signaling in cancer cells. Interestingly, naturally occurring phytochemicals found in human diets (either singly or as mixtures) may promote anti-growth signaling, and do so without the potentially adverse effects associated with synthetic chemicals. We review examples of naturally occurring phytochemicals that may be applied to prevent cancer by antagonizing growth signaling, and propose one phytochemical for each pathway. These are: epigallocatechin-3-gallate (EGCG) for the Rb pathway,

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luteolin for p53, curcumin for PTEN, porphyrins for Hippo, genistein for GDF15, resveratrol for ARID1A, withaferin A for Notch and diguelin for the IGF1-receptor pathway. The coordination of anti-growth signaling and natural compound studies will provide insight into the future application of these compounds in the clinical setting.

1. Introduction

Carcinogenesis is a complex, stochastic and yet highly coordinated multi-step process in which normal cells progress through hyperplasia to mild, moderate and severe dysplasia to carcinoma *in situ*, invasive carcinoma, and finally to metastatic disease after initiation by primary carcinogenic insult [1]. Hahn and Weinberg [2] proposed six hallmarks to better define and understand this complex process. They modeled these hallmarks in normal human bronchial epithelial cells and demonstrated immortalization *in vitro* by targeting tumor suppressor pathways, notably, retinoblastoma (*Rb)* regulation of cell cycle entry, tumor protein 53 (*TP53)* regulation of cell cycle progression, human telomerase reverse transcriptase (*hTERT)* activation, combined with an oncogenic signal using activated Harvey rat sarcoma viral oncogene homolog (*hRAS*) [3]. As this model shows, and as studies of human tumors progress into the era of high throughput sequencing, it is clear that evasion of anti-growth signaling and loss of tumor suppressors are central hallmarks necessary to the oncogenic process.

Loss of growth control mechanisms allows neoplastic cells to acquire unlimited replicative ability and evade elimination, growth arrest, and senescence by tumor suppressors. In general, tumor suppressor genes block the transformation of normal cells to cancerous cells. Environmental stress factors including ultraviolet (UV), irradiation, and chemicals can induce DNA damage and genetic alteration. These injuries can cause the progression of carcinogenic processes if damage cannot be appropriately repaired and mutated cells continuously proliferate. Dozens of tumor suppressor genes are activated under these circumstances that inhibit the proliferation of damaged/mutated cells by arresting cell cycle progression and inducing apoptosis and other types of programmed cell death, thus their evasion is critical for carcinogenesis. p53 and Rb are typical tumor suppressor genes [4]; they play a key role in determining the fate of cells, *i.e.* whether they proliferate or undergo senescence or apoptotic programs. In solid tumors, the most common genetic changes are losses of tumor suppressor genes. It has been estimated that over 70% of the genetic changes discovered in solid tumors represent evasion of tumor suppressor mechanisms; leading to the suggestion that this leaves us with an un-targetable cancer problem. It would appear necessary to replenish the function associated with the mutated or lost tumor suppressor in every tumor cell, a goal that has so far been unattainable. However, loss of a tumor suppressor usually results in unopposed signaling by a mechanism normally suppressed by the lost tumor suppressor gene. Thus, a viable strategy to overcome the evasion of a tumor suppressor mechanism is to identify and target the unrestrained pathways activated by the loss of tumor suppressors.

This review will briefly discuss how anti-growth signaling mechanisms are inactivated in tumors with emphasis on major tumor suppressor pathways and will explore how these pathways can be targeted for the prevention and treatment of cancer.

2. Dysfunction: mechanism of evasion of tumor suppressors

Tumor cells may evade tumor suppressors by genetic and epigenetic mechanisms. Genetic mechanisms include chromosomal deletion, mutation and inactivation or loss of upstream or downstream effectors. Epigenetic evasion includes DNA methylation, and histone methylation and acetylation. Examples of tumor suppressor losses are abundant in solid tumors. Among the most common are loss, mutation and/or methylation of the cyclin-dependent kinase inhibitor (CDKN) *2A* locus on chromosome 9p21, which leads to loss of the CDKN, *p16ink4a* and often the mouse double minute 2 homolog (hMDM2) inhibitor $p14^{ARF}$ as well. Loss of *p16ink4a* results in unopposed activation of the cyclin dependent kinases *CDK4/6*, which phosphorylate the Rb protein thereby activating E2F-mediated transcription of genes involved in entry into the cell cycle. Loss of p14ARF protein results in unopposed MDM2 activity and increased p53 ubiquitination and degradation with effects similar to loss of p53. Mutation, loss or inhibition of *TP53* function is also very common, as is loss and/or mutation of phosphatase and tensin homolog (*PTEN).* Loss of p53 leads to loss of cell cycle checkpoints, the ability of the cell to arrest and effectively repair DNA errors or damage and the accumulation of genetic instability and accumulation of mutations. Additionally, p53 protein has an important role in triggering apoptosis, thus its loss leads to the inappropriate survival of cells with new mutations. Loss of PTEN protein, a phosphatase that dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate (PIP3), leads to unopposed activity of phosphoinositide 3-kinase (PI3K) and protein kinase B (AKT) signaling, which drives tumor growth.

The dysfunctional pathways activated by loss of tumor suppressors provide continuous unopposed tumor growth promoting signals. These pathways have consequently become potential targets for novel anti-cancer compounds. For example, inhibitors of MDM2 are being tested to restore p53 function, mammalian target of rapamycin (mTOR) inhibitors are being tested to overcome PTEN loss, and CDK4/6 inhibitors to restore Rb function from p16ink4a loss of function are entering clinical trials.

3. Prioritized anti-growth signaling pathways

There are hundreds of tumor suppressor genes that possess the ability to stop or slow down the carcinogenesis process. The activation of tumor suppressors is mostly context-dependent and varies by organ site and by molecular and pathological sub-type. The most common and important tumor suppressors, their role in tumorigenesis and approaches to target these genes for cancer treatment and prophylaxis are discussed in the following sections and summarized in Fig. 1.

3.1. The Rb pathway

The retinoblastoma (*Rb*) gene was the first tumor suppressor gene to be described. The development of retinoblastoma was predicted by Alfred Knudsen to involve a “two hit” mechanism, based on the kinetics of appearance of retinoblastoma in the inherited form (single order kinetics) and the sporadic form (second order kinetics). This analysis led to the hypothesis that disease initiation requires two steps involving loss of function of both copies of the affected gene. Thus, Rb was recognized to have a tumor suppressor function long before the gene was identified and demonstrated to be inactivated by mutation of one copy and loss or silencing of the