Molecular targets for chemoprevention: Role in choosing and developing new agents

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Introduction

The field of cancer chemoprevention has developed considerably over the past two decades with several large randomised trials having been undertaken. Some of these have been positive and have included a demonstration of the efficacy of tamoxifen to reduce the risk of breast cancer [1] and finasteride to have an impact on prostate cancer incidence [2]. Unfortunately several trials have been negative and some have even produced results suggesting a deleterious effect of the agent under investigation [3]. Chemoprevention trials are, of necessity, usually very large and extremely costly. To date, one of the main methods for choosing agents to investigate in trials has been retrospective population-based analysis of an apparent association between ingestion of the agent and a reduced incidence of cancer. Unfortunately such analyses are often flawed by many sources of bias. In addition, adverse effects of exposure to such agents over a protracted period have not been anticipated. Successful chemoprevention requires an agent which has a significant impact on cancer risk but is also extremely safe and well tolerated. With the increasing understanding of molecular mechanisms leading to carcinogenesis, it is imperative that cancer preventive agents are developed more rationally and are targeted towards these mechanisms – both to increase efficacy and also to reduce the potential for toxicity. This article will highlight some of the more promising potential targets which can be used to choose and design drugs for testing as chemopreventive agents. The identification of targets could also enable the development of biomarkers to test efficacy during early clinical development.

Molecular targets for chemopreventive agents

Phenotypic and genotypic alterations occur during carcinogenesis and lead to the features which define a malignant cell. One of these involves abnormalities in the survival pathways which enable malignant cells to avoid apoptosis. Among the best described survival pathways are those involving the growth factor receptor (GFR)/Ras/Mitogen Activated Protein Kinase (MAPK) pathway [4], phophotidylinositol-3-kinase (PI3K)/protein kinase B(Akt) [5] and signal transducers and activators of transcription (STATs) [6] or nuclear factor κB(NF-κB) [7].

Signalling through the GFR/Ras/MAPK pathway is central to regulating cell growth and several growth factors activate the pathway. In many malignancies there is amplification or over expression of GFRs and oncogenic Ras leading to continuous activation of the pathway. The PI3K/Akt pathway appears to be central to survival of malignant cells with activation blocking a number of mechanisms that would normally induce apoptosis or lead to cell cycle arrest in damaged cells. Malignant cells are frequently associated with over expression or increased activation of different components of this pathway. The transcription factor NF-κB has been increasingly studied in recent years and appears to be central to promoting tumour cells in malignancies which are associated with inflammation. Normally, NF-κB is associated with IκB which leads to its inactivation. During malignancy, IκB can be inactivated and degraded, freeing NF-κB to translocate to the nucleus where it can promote transcription to several different genes that lead to induction of proliferation and inhibition of apoptosis. There has been increasing demonstration of deregulation of this pathway in malignancy. The STATs pathways are involved in the regulation of gene transcription in response to cytokine or growth factor stimulation of a variety of receptors. They appear to become overactive in several malignancies. This can lead to the prevention of apoptosis through increased expression of anti-apoptotic proteins such as Bcl2 and Bclx1.

Cell cycle regulation

One of the hallmarks of malignancy is a loss of regulation of cell cycle progression. Several proteins have been identified which regulate progression through the cell cycle and a group of molecules, the cyclins and cyclin-dependent kinases have been extensively investigated in recent years. Cyclin D1 is a rate-limiting factor in progression of cells through the G1 phase of the cell cycle [8]. Its over expression is associated with carcinogenesis – particularly in breast and prostate cancer. Another target protein involved with cell cycle progression is activator protein-1 (AP-1) [9]. A variety of growth factors and oncoproteins are potent inducers of AP-1 activity. AP-1 appears to be involved in the regulation of genes involved with apoptosis and cell cycle progression by activating the cyclin D1 gene. It also has effects at repressing tumour-suppressor genes including p53. It may also have a role in controlling metastasis.

The tumour suppressor gene, p53, provides another potential target for chemoprevention. It regulates many cell processes including signal transduction, responses to DNA damage, cell cycle control and apoptosis. p53 mutations are found in most tumour types and appear to have numerous roles in carcinogenesis.

Growth factor signalling pathways

Many growth factors have been implicated in carcinogenesis and include epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF)-α and -β and insulin like growth factor (IGF). Many tumours appear to progress as a result of increased activation of downstream signalling from activation of the receptors. These result in increased cell proliferation and suppression of apoptosis together with an increased potential for invasion and metastasis. Agents targeted at the EGF receptor (EGFR) may result in apoptosis of cancer cells.

Cyclo-oxygenase 2 (COX-2)

The cycloxygenases (COX-1 and COX-2) have been an increasing focus as targets for chemopreventive drug development [10]. These enzymes are responsible for converting arachadonic acid into prostatglandins. COX-2 is overexpressed in almost every pre-malignant and malignant cell. Several mechanisms associated with the activity of COX-2 in neoplasia have been defined in recent years and these include induction of resistance to apoptosis, promotion of angiogenesis, increase in DNA mutagenesis and increased invasiveness and reduced adhesion of epithelial cells.

Use of molecular targets to identify chemopreventive agents

An understanding of the multiple molecular pathways on targets which are deregulated during carcinogenesis enables *in vitro* and *in vivo* assays to be developed to screen potential chemopreventive agents before further development occurs. An understanding of the importance of increased expression of COX-2 has led to the *in vitro* testing of several agents for their effects on COX-2 expression. Modulation of this enzyme increases the potential interests of an agent as would measured effects on cell proliferation and survival and markers of apoptosis. Several groups have also developed assays of DNA damage and modification of the level of damage by potential chemopreventive agents may further add to their interest for future development. Such *in vitro* assays clearly have limitations but, positive results taken together with effects on animal model systems (e.g. transgenic or carcinogen-induced models of malignancy) can provide sufficient rationale for the development of trials in humans. Of increasing importance is the development of biomarkers of efficacy in human studies. Chemoprevention trials which use cancer incidence as an endpoint require prolonged follow up and large numbers of individuals. The use of surrogate biomarkers which may involve modification of molecular targets of carcinogenesis can be measured in humans before and after exposure to a new potential chemopreventive agent and can give much earlier indications of potential efficacy.

The field of chemoprevention is thus beginning to benefit from the incorporation of an understanding of molecular targets for drug development. The increasing incorporation of the understanding of such molecular targets should markedly improve the ability to identify appropriate agents for trials and should speed up the determination of clinical efficacy in the future.

Conflict of interest statement

None declared.

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