Inhibition of ras Oncogene: A Novel Approach to Antineoplastic Therapy

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Abstract
The most frequently detected oncogene alterations, both in animal and human cancers, are the mutations in the ras oncogene family. These oncogenes are mutated or overexpressed in many human tumors, with a high incidence in tumors of the pancreas, thyroid, colon, lung and certain types of leukemia. Ras is a small guanine nucleotide binding protein that transduces biological information from the cell surface to cytoplasmic components within cells. The signal is transduced to the cell nucleus through second messengers, and it ultimately induces cell division. Oncogenic forms of p21ras lead to unregulated, sustained signaling through downstream effectors. The ras family of oncogenes is involved in the development of both primary tumors and metastases making it a good therapeutic target. Several therapeutic approaches to cancer have been developed pointing to reducing the altered gene product or to eliminating its biological function: (1) gene therapy with ribozymes, which are able to break down specific RNA sequences, or with antisense oligonucleotides, (2) immunotherapy through passive or active immunization protocols, and (3) inhibition of p21ras farnesylation either by inhibition of farnesyl transferase or synthesis inhibition of farnesyl moieties.

It is widely accepted that neoplastic diseases have a genetic origin. Cancer can result from DNA damage, caused by physical, chemical or biological carcinogens, or spontaneously during DNA replication. DNA damage can lead to cancer in two distinct ways: an altered gene encoding for a protein that brings about unrestricted cell growth, or alternatively, a damaged gene which can no longer code for a protein needed for normal growth arrest [6]. In other words, too much stimulation, not enough blockade, or both simultaneously can lead to tumor formation. The damaged genes that stimulate cell growth are known as oncogenes [5], and those that halt growth are known as tumor suppressor genes or antioncogenes [54]. The aforementioned genetic alterations lead to a disruption in the homeostatic equilibrium of cell growth, which dictates that in an adult organ or tissue, the number of newly generated cells must equal the number of disappearing cells. The homeostatic interaction between positive and negative signals is frequently seen in biology.
This equilibrium is lost in cancer, and mutations in both oncogenes and tumor suppressor genes contribute to the malignancy.

The establishment of the 'oncogene theory' by Huebner and Todaro [22] in 1969 and the following development of molecular biology, which has increased extraordinarily during the past decade, have made it possible to study many tumors at the molecular level. Studies performed on neoplastic cells from tumors coming from different species of laboratory animals [46, 20], from humans [40, 29], and from cultured cell lines [45, 12] have gone a long way towards revealing the genetic alterations that lead to the transformed state. Genetic alterations can result from mutation [18], amplification [14], translocation [55], or other genetic mechanisms [38]. Simultaneous alterations in oncogenes and tumor suppressor genes can be detected in most tumors [4]; only a few examples are known where a single gene is altered. DNA damage is increased during the tumor progression [37]. In some tumors, such as colon adenocarcinoma, the exact sequence of gene alteration events leading to tumor development is known [15].

Studies at the molecular and cellular level of different tumors in diverse experimental models have enabled researchers to postulate new therapeutic strategies that, through distinct approaches, aim to revert or stop neoplastic growth. This can be achieved by (1) restoring the normal levels of an altered or absent gene product (for example, a tumor suppressor gene product) [31, 53], or (2) reducing a detrimental effect by inhibiting the synthesis of the altered gene product, or by abolishing its biological function [44, 47]. Both oncogenes and tumor suppressor genes are phylogenetically conserved, a fact that indicates the vital importance of their functions [32]. Furthermore, all vital functions are protected by the existence of redundant mechanisms that ensure their proper performance. Considering this, the success of a therapeutic strategy aimed to a single gene would seem unlikely. Nevertheless, different clinical trials are based on its use, particularly in conjunction with other therapies intended for correcting other aspects of the functioning of the cell.

The most frequently detected oncogene alterations, both in animal and human cancers, are the mutations in the ras oncogene family [8]. These oncogenes are mutated and/or overexpressed in many human tumors, with a high incidence in pancreatic carcinoma (90%), thyroid and colon cancer (50%), lung cancer (30%), and myeloid leukemia (30%).

The ras oncogene family encodes for proteins with a molecular weight of 21 kd, known as p21 ras. These proteins are associated with the cytoplasmic side of the plasma membrane, where they participate in the transduction pathway of signals generated on the outer side of the plasma membrane. The signal is transduced to the cell nucleus through second messengers, and it ultimately induces cell division, either directly or indirectly [23]. In other words, Ras proteins act as central linkers between signals generated on the cell surface by growth factors or cytokines, and nuclear effectors (fig. 1).

The disruption of signal transduction cascades involving Ras proteins could constitute a potential antineoplastic strategy, not only in tumors carrying a mutated form of ras, but also in those where the signal transduction pathway is deregulated through growth factors or their receptors [30].

p21 ras, either mutated or not, has to be associated with the plasma membrane in order to carry out its biological function. This anchorage is achieved through a farnesyl group that modifies a cysteine residue present in a CAAX motif, near the C-terminus of the Ras precursor. The post-translational farnesylation is catalyzed by farnesyl transferase (FT). The absence of a farnesyl group on p21 ras prevents its association with the plasma membrane, and consequently, it inhibits its function [28].

The ras family of oncogenes is involved in the development of both primary tumors and metastases [7, 10], making it a good therapeutic target. Normal cell growth depends on several growth factors, and each one of them activates several intracellular signaling pathways. Consequently, the inhibition of a single transduction event generated by a mutated or overexpressed oncogene in a tumor cell will have an effect on the tumor cell only, and not on normal cells. Most cancers with an alteration on the ras oncogene are chemoresistant and radioresistant, therefore they cannot be treated with standard therapies. For this reason, new treatments are being developed, using novel therapeutic strategies and methodologies (fig. 2).

Gene Therapy

Gene therapy is one of the most revolutionary therapeutic procedures of the last decade [3]. The goal of this therapy is to specifically correct the molecular damage causing a given pathology. In cancer, gene therapy aims at the elimination of mutated or overexpressed genes, or the products they code for. One of these strategies employs ribozymes, which can inhibit gene expression [41]. Ribozymes are RNA molecules with enzymatic properties that