New targets for tumor radiosensitization

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INTRODUCTION

Targeting of radiotherapy to cancer cells and avoiding irradiation of normal tissue plays a central role in improving the efficacy of radiotherapy. Biological targeting requires a systemically given compound that interacts with radiation. The degree of targeting is critically dependent on the specificity of the metabolic process used. Over the last few years, there has been an explosion in the understanding of cellular, biochemical and molecular effects of ionizing radiation in eukaryotic cells. Many of the genes encoding proteins involved in repairing radiation damage have been identified and cloned. These proteins are potential products for modifying the radiation response of normal and malignant tissues. Although our knowledge of these signaling pathways is quite rudimentary, we can now begin to explore how some of these tumor genes or protein products may be targets for modifying the ionizing radiation response in human cancer. A number of agents directed against these products have been produced. In vitro studies have shown the effectiveness of this strategy and phase I-II clinical trials have yielded promising results.

However, a lot of work has yet to be done to overcome a variety of challenges. Any pharmacological intervention has to be specific in order to increase effectively the radiation therapeutic ratio. Such specificity for tumor or normal tissue can be obtained by exploiting molecular differences between normal and malignant cells, but cell kill depends on multifactorial mechanisms and exactly how the blockage of a specific pathway may affect radiation tumor response in human malignancies is yet to be addressed. Clinical research has to define how these new agents are integrated with radiotherapy and chemotherapy. Indeed, novel targeting approaches for tumor radiosensitization is an evolving area of active translational and clinical research in radiation oncology. This review provides an update on the status of some of these novel targets for human tumor radiosensitization.

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RADIATION RESPONSE FOLLOWING INHIBITION OF EPIDERMAL GROWTH FACTOR RECEPTOR

The epidermal growth factor receptor (EGFR) is a 1.186-amino acid, 170-kilodalton transmembrane glycoprotein and member of the family of type 1-receptor tyrosine kinases. The EGFR is composed of an extracellular ligand-binding domain, a transmembrane lipophilic segment, and an intracellular protein kinase domain with a regulatory segment. After ligand binding, EGFR dimerization occurs, which produces activation of the intrinsic protein tyrosine kinase activity. This leads to the activation of a cascade of biochemical and physiological responses involved in the mitogenic signal transduction which initiate in the cytoplasm and reach the nucleus, eventually prompting mitogenesis. A primary function of EGFR revolves around its capacity to influence cellular growth, proliferation and differentiation. A variety of ligands for the EGFR have been identified. These include epidermal growth factor (EGF), transforming growth factor alpha (TGFα), amphiregulin, and heparin-binding EGF.

All cells of epithelial origin as well as many cells of mesenchymal derivation express EGFR. There are several reports suggesting that EGFR is often expressed at high levels in human cancer and has been associated with more aggressive tumors. Indeed, a correlation between EGFR expression and clinical stage, disease progression, response to therapy, and patient survival has been found in the most common human malignances. Signaling through EGFR may increase the resistance to radiation and chemotherapy, and blocking EGFR pathway could sensitize these agents. Interruption of EGFR activation can be accomplished through the use of anti-EGFR monoclonal antibodies, which block the binding of endogenous ligands, or by the use of pharmacological agents that inhibit EGFR phosphorylation, such as tyrosine kinase inhibitors.

C225 or cetuximab is a chimeric monoclonal antibody directed against the extracellular ligand-binding domain. The combination of cetuximab and

radiation has been evaluated using in vitro and in vivo models. In vitro studies have shown that cetuximab inhibits tumor cell growth kinetics (G1 arrest), enhances cellular radiosensitivity, and promotes radiation-induced apoptosis. Human squamous cell carcinoma xenografts in athymic mice, treated with cetuximab and radiation, have resulted in complete tumor regression. These results have been confirmed by other investigators and support the finding that cetuximab is a potent radiation enhancer. However, the main endpoint for blocking EGFR in fractionated radiotherapy is to inhibit cellular proliferation during radiotherapy. Harari has postulated the following mechanisms: inhibition of DNA damage repair, enhancement of radiation-induced apoptosis, inhibition of tumor angiogenesis and effects on tumor cell migration and invasion capacity.

These promising results have led to the incorporation of cetuximab in clinical trials for the treatment of a wide variety of human malignances such as head and neck, colorectal, pancreatic or lung tumors. Bonner conducted a phase I/II trial with the aim to evaluate the safety, pharmacokinetics, and efficacy of cetuximab, in combination with radiation therapy in patients with advanced squamous cell carcinoma of the head and neck. He treated 16 patients with conventional or hyperfractionated radiotherapy and cetuximab. A loading dose of 100 to 500 mg/m^2 was employed, followed by weekly infusions of 100 to 250 mg/m^2 during radiotherapy. The toxicity profile was moderate and complete response rate was achieved in 80% patients. The recommended dose for phase III studies is a loading dose of 400 mg/m^2 and a maintenance weekly dose of 250 mg/m^2. Recently, it has been finished a phase III trial comparing radiation alone versus radiation with cetuximab in locally advanced head and neck cancer, which has enrolled more than 420 patients. Cetuximab have been also combined with cisplatin in head and neck cancer patients. Platinum-refractory patients were treated with cisplatin/carboplatin and cetuximab. Ninety-six patients were enrolled and a 14.6% response rate was obtained, showing that cetuximab plus platinum has activity in platinum-refractory patients. To date, cetuximab seems to be well tolerated and adverse effects were moderate. The most commons are fever, allergic reactions and follicular rash (fig. 1) that resolve spontaneously after cessation of cetuximab.

ZD1839 or iressa is a selective inhibitor of the EGFR-tyrosine kinase, which is directed against the cytoplasmic protein tyrosine kinase domain of the EGFR. Iressa is a quinazoline, orally available,

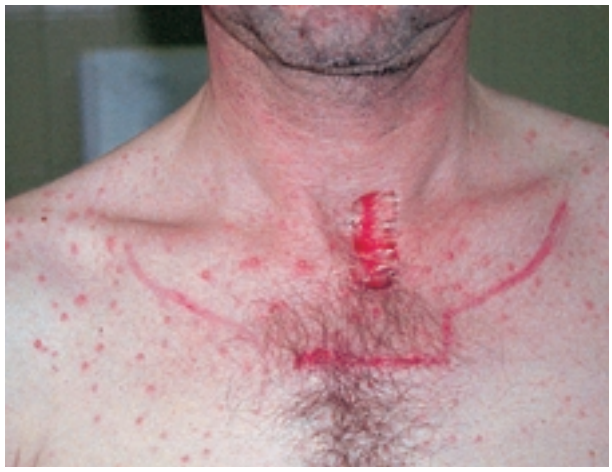

Fig. 1. Grade 1 follicular skin reaction of a patient treated with cetuximab and radiotherapy for advanced head and neck carcinoma.

which inhibits ligand-induced cell growth by competitive inhibition of ATP. Complete regression of well established human carcinoma xenografts in nude mice have been achieved. Preclinical studies confirm the capacity of iressa to enhance radiation in a spectrum of human cells lines including lung, pancreas and head and neck. Although clinical trials have focused on iressa alone or in combination with conventional chemotherapy, in vitro data suggests the association of iressa and radiotherapy needs to be evaluated in clinical trials. Other tyrosine kinase inhibitors such as OSI-774 (tarceva) or CI-1033 have been evaluated in conjunction with radiotherapy in preclinical studies.

Molecular modulation of the EGFR pathway offers a new tool in the treatment of cancer. Different compounds have proven to successfully block EGFR. As clinical trials mature regarding the use of EGFR-inhibitory agents in combination with radiation and chemotherapy, the role of EGFR targeting in the treatment of cancer will be defined.

CYCLOOXYGENASE-2 ENZYME INHIBITORS

Cyclooxygenase-2 (COX-2) is an enzyme induced by a variety of factors including tumor promoters, cytokines, growth factors and hypoxia. It has been involved in the metabolic conversion of the arachinodic acid to prostanoids, primarily in inflammatory states and tumors. Research suggests that the COX-2 enzyme is overexpressed in a broad range of premalignant, malignant, and metastatic human epithelial cancers. COX-2 plays a major role in colon carcinogenesis. The enzyme is detected in 40-50% of malignant adenomas and in more than 80% of adenocarcinomas. The COX-2 enzyme is also expressed in existing and angioge-

nic vasculature within and adjacent to hyperplastic/neoplastic lesions. Besides their involvement in tumor development, COX-2 and its catalyzed products have been implicated as regulators of tumor growth rate and tumor dissemination. COX-2 is overexpressed in a broad range of human malignances, including colon, breast, head and neck, lung and pancreatic cancers.

Treatment with selective COX-2 inhibitors such as nonsteroidal anti-inflammatory drugs (NSAID), frequently reduces tumor growth and metastases produced by COX-2. The development of colon tumors is reduced with COX-2 blockage in mice. Indeed, indomethacin lowered prostaglandin levels in mouse tumors and significantly increased tumor radioresponse, which was manifested in prolonged tumor growth delay. The mechanisms underlying this retardation are: inhibition of tumor cell proliferation, induction of apoptosis, inhibition of neoangiogenesis and stimulation of antitumor immune response.

In vitro clonogenic cell survival assays have shown a direct action on tumor cells killing by radiation enhancement with selective COX-2 inhibitors. NSAID or selective COX-2 inhibitors such as EGFR kinase may affect a number of molecules regulating cell survival.

Study of the biology of COX-2 and the role of this enzyme and its products in tumor growth and response to radiation is rapidly evolving. The information on the ability of selective COX-2 inhibitors to enhance radiotherapy is limited to preclinical setting but these agents may have significant therapeutic potential.

INCREASING RADIOSENSITIVITY WITH RAS INHIBITORS

Over the last 15 years there is experimental evidence showing that cells transformed with the activated Ras proto-oncogene are more radioresistant. The Ras proto-oncogenes encode a protein that regulates cellular growth and differentiation. There are 3 proto-oncogenes, H-Ras, K-Ras and N-Ras, which encode 4 proteins: H-Ras, N-Ras, K-Ras4A and K-Ras4B. The Ras proteins are signal transducers from growth factor receptors to downstream effect molecules such as members of the MAP kinase family. The molecule acts as a switch that is inactive when bound to guanine diphosphate (GDP) and active when combined with guanine triphosphate (GTP). Activation of growth factor receptors by their ligands induces its transphosphorylation which promotes activation of Ras, through the formation of its GTP-bound complex. Ras mutations can lead to a protein that is permanently active, providing a mitogenic signal in the absence of growth factors. Such mutations are powerful oncogenic stimuli and are detected in about 30% of human malignancies. To be active, Ras needs to be located at the inner surface of cell membrane. This is achieved by either farnesylation of the last cysteine of the protein or by an arginine rich region at the carboxy terminal. Farnesylation involves the addition of a 15-carbon farnesyl isopreniod in the cystein residue.

Several agents that block Ras function by interfering with protein farnesylation have been synthesized. These farnesyltransferase inhibitors (FTI) can cause reversion of the Ras transformation and inhibit the growth of Ras-transformed fibroblasts and human tumor cells, in vitro. FTI have a good toxicity profile and appear to lack growth-inhibitory activity against nonmalignant cells. Several FTI have shown to reverse cellular resistance to radiation in mutant Ras-containing cells lines. Inhibition of Ras farnesylation using the FTI-227 increases the radiosensitivity in Ras-transformed cells, but does not influence the radiation sensitivity of normal fibroblasts or tumor wi-thout an activating mutation in Ras. Radiosensitiz a t i o n by FTI has also been shown in vivo. Cohen evaluated the use of FTI in nude mice bearing T24 tumor cell xenografts. Treatment with FTI L-774632 resulted in a significant reduction in tumor cell survival after radiation compared with controls. This effect was limited to tumors expressing mutated Ras and not observed in tumors with wild type Ras.

Early clinical studies are under way to test FTI combined with chemotherapy and/or fractionated radio-therapy advanced cancers. A number of FTI agents such as R115777, BMS-214662, SCH6636 or L-778123, have been evaluated in phase I and II. L-778123 has been administered in combination with radiotherapy. Twenty-one patients with pancreatic, lung, and head and neck cancers were enrolled. Radiotherapy doses ranged from 60 to 70 Gy. Dose-limiting toxicities included grade 3 diarrhea and grade 4 hematological. L-778123 appears to be well tolerated and responses have been reported in non-small-cell lung cancer and head and neck tumors. These results, warrants futures studies.

TUMOR ANGIOGENESIS

Angiogenesis is a fundamental requirement for new organ development and for differentiation during embryogenesis, wound healing, and reproductive functions in adults. Also takes place in some pathologic conditions, such as rheumatoid arthritis, age-related macular degeneration, pro-

liferative retinopathies, and tumor growth and metastasis. In 1971, Folkman proposed that tumor growth and metastasis are angiogenesis dependent, and hence, blocking angiogenesis could be a strategy to arrest tumor growth. Angiogenesis is a complex process that is tightly regulated by pro and anti-angiogenic growth factors. Some of these factors are highly specific for the endothelium (vascular endothelial growth factor or VEGF), while others have a wide range of activities (matrix metalloproteinases or MMPs). Angiogenic growth factors are released in response to hypoxia, growth factors such as EGF, FGF, or interleukins, inflammatory proteins (COX-2 or prostaglandins), mechanical stress (pressure caused by proliferating tumor cells), and genetic alterations. The released growth factors bind to, thereby activating, endothelial cells that form the walls of nearby blood vessels.

Activated endothelial cells transduce this signal to their nucleus to produce enzymes, such as matrix metalloproteinases (MMPs). These enzymes break down the extracellular matrix of the blood vessel, allowing endothelial cells to invade the matrix and to divide in response to tumor-derived growth factors. The proliferating endothelial cells migrate through the holes made by the enzymes toward the growth factor stimulus. Adhesion molecules or integrins mediate the migration of the new endothelial cells toward the growth factor stimulus and additional enzymes are released to dissolve the surrounding tissue. The adhesion receptor integrin v3, present on the surface of activated endothelial cells, is required for the differentiation, maturation, and survival of blood vessels. Strings of new endothelial cells organize into hollow tubes, thus creating new blood vessels, and individual blood vessels connect to form vessel loops or networks that allow blood to circulate. Structural support is provided to the new blood vessels by pericytes. The vessels are then ready to carry blood to the tissue that initially released the pro-angiogenic growth factors.

Tumors require angiogenesis to grow and to facilitate metastasis. VEGF plays a central role in tumor angiogenesis; it is expressed in most tumors, often at substantially increased levels. VEGF is stimulated by hypoxia, by oncogenes such as Ras or by cytokines. VEGF expression is associated with tumor growth, angiogenesis, metastasis, and poor outcome.

VEGF and its receptors are good targets for cancer therapy. Several strategies have been used to inhibit VEGF, including anti-VEGF monoclonal antibodies, coupling a toxin to VEGF, soluble VEGF receptors, peptides that interfere with VEGF binding, and agents that block VEGF receptor signaling. A number of compounds have been studied in clinical trials.

SU 5416 (Semaxanib) is a novel synthetic compound, a specific VEGF receptor (Flk-1) antagonist that decreases VEGF-stimulated Flk-1 phosphorylation. Because it is a specific angiogenesis antagonist, SU5416 does not directly inhibit tumor cells in vitro. However, SU5416 shows broad antitumor efficacy in subcutaneously implanted tumor xenografts in athymic mice. In a phase I study, 69 patients with advanced malignancies were treated. Dose-limiting toxicities were reversible grade 3 headaches, nausea, and grade IV vomiting. Bevacizumab, a RhuMAb-VEGF (Avastin) has shown reduction of VEGF levels, selective inhibition of VEGF receptor 2 (KDR/Flk-1), and blocks tumor growth in mice. In phase I studies, bevacizumab has been safely administered alone and in combination with chemotherapy. Bevacizumab efficacy was subsequently evaluated in a randomized phase II study enrolling 104 patients with previously untreated metastatic colon cancer with promising results.

Several groups have examined the interaction between anti-VEGF and radiation, and found a significantly tumor growth inhibition. Overall, these findings suggest that introducing an angiogenesis inhibitor may enhance the radiation antitumor effect. Phase I studies of this combination are under way.

Many molecular processes and signaling pathways were identified. These cellular and molecular pathways are available for radiation modification by chemical and biologic compounds, providing new opportunities for translation research in radiation oncology and for more effective combined-modality treatment of cancer. Our improved understanding of these targets will undoubtedly generate additional new anticancer agents in the future.

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