The Radiosensitive Therapy for Colorectal Cancer

Zifa Wang¹,2, Tracy Cook¹, David Blumberg¹

1. Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA 15232, the United States
2. Orient Organ Transplantation Center, Tianjin First Central Hospital, Tianjin 300192, China

Abstract: The colorectal cancer is one of the most common cancers in the United States. Surgical resection is appropriate as the first component of treatment. However, only less than half of the patients are cured by primary resection. Neoadjuvant radiation can decrease tumor size before surgery, and can enable patients previously deemed unresectable, to undergo curative surgical resection. Unfortunately since many tumors have hypoxic areas or genetic mutations that enable them to be radioresistant, only 22 - 44% of rectal cancers will respond to neoadjuvant radiation. To overcome the radioresistance of tumors, multiple drugs have been tested as potential radiosensitizers. 5-Fluorouracil is one of the most widely used radiosensitizers, which enhances radiosensitization of colorectal cancer due to alteration of cell kinetics. Nitric Oxidize is a promising potential radiosensitizer. AdiNOS treatment of HCT-116 tumors significantly delayed tumor doubling time and growth when combined with single or multifractionated radiation. Other radiosensitizers, such as, Protein Kinase C-specific Inhibitor PKC412, Survivin, Caffeine, Bromodeoxyuridine and Iododeoxyuridine are also briefly discussed in this review. [Life Science Journal. 2005; 2 (1): 55 - 60] (ISSN: 1097 - 8135).

Keywords: colorectal cancer; neoadjuvant radiation; radioresistance; radiosensitizers

1 Introduction

The colorectal cancer is the third leading cause of cancer in the United States with approximately 150,000 new cases annually. The primary modality for treatment for colorectal cancer is surgical resection. For every 100 patients initially evaluated, 45 are cured by primary resection[1]. Even though remarkable progress has been made in the treatment of the colorectal cancer during the past two decades, approximately 44% of patients with colon cancer will present with stage III or IV disease[2]. Neoadjuvant radiation can decrease tumor size before surgery, enabling a greater chance of obtaining a tumor-free surgical margin and can enable patients previously deemed unresectable, to undergo curative surgical resection[3]. Several studies have examined preoperative irradiation alone as a neoadjuvant regimen for the treatment of rectal cancer. The European Organization for Research and Treatment of Cancer studied randomized patients with T2 - T4 tumors to preoperative radiation or surgery alone. Although the radiation dosage of 3,450 cGy was lower than the standard dosage given today, there was a 50% reduction in the local recurrence rates in patients treated with radiation[4]. The first prospective, randomized controlled study documented that preoperative therapy reduces local recurrence and improves survival[5]. Despite these benefits, unfortunately only 22 - 44% of rectal cancers will respond to neoadjuvant therapy[6]. To overcome the radioresistance of tumors, multiple drugs such as, fluorodeoxyuridine, caffeine, and nitric oxidize have been tested as potential radiosensitizers[7 - 9]. In this article, we review some potential radiosensitive agents in colorectal cancer.

2 Molecular Mechanisms of Radiosensitization

Radiation is considered to have ionizing potential. The radiation used in radiation therapy (RT) produces several hundred thousand ionization events per cell per gray. The absorbed energy causes ejection of primary electrons that go on to ionize other molecules leading to a complex chain reaction. DNA is the most important target for RT. The evidence from experiments shown that irradiation of the nucleus, but not the cytoplasm, results in cell death. The extent of initial DNA damage and the persistence of this damage are presumably critical factors in determining the cellular response to radiation. In the process, free radicals, which are neutral atoms or molecules that have an unpaired electron, are generated. Because of their unpaired electrons, free radicals are very reactive and can reduce or oxidize biological molecules and break their chemical bonds. Since the most abundant molecule in cells is water, the most common free radicals
that are generated in a cell after exposure to ionizing irradiation are reactive oxygen species (ROS). Biological agents, such as growth factors, cytokines, monoclonal antibodies to cell surface receptors, can alter molecular pathways within a cell. Chemotherapeutic and physical agents, such as hyperthermia, hypoxia and radiation itself, can activate pathways that can also affect the outcome.

An important consequence of the involvement of free radicals in radiation damage is that oxygen plays a major role as a modifier of radiation responses. Oxygen influences the nature of the free radicals and the lesions that are formed. The peroxides and hydroperoxides, in particular, inhibit repair. Under hypoxic conditions the cells are typically 2.5 - 3 times more resistant to irradiation than in the presence of oxygen\(^{10}\). A number of studies have documented the importance of hypoxia to the outcome of RT. At the molecular level, hypoxia induces expression of a number of genes, in particular genetic programs that are under the control of hypoxia inducible factor (HIF-1). Some of these (such as erythropoietin, vascular endothelial growth factor and tumor necrosis factor alpha (TNF-\(\alpha\))) are clearly aimed at increasing angiogenesis and increasing oxygen delivery. Hypoxia can therefore play an important role in driving angiogenesis and tumor expansion. Other hypoxia-induced genes (such as \(p53\)) are part of a stress response that encourages cells to undergo cell death by apoptosis. Acting in this way, hypoxia serves as a selective force to favor expansion of cells mutated in \(p53\)\(^{11}\).

Expression or knockout of proto-oncogenes, tumor-suppressor genes, cytokines, cytokine receptors, cell adhesion molecules, redox-active genes and many other genes that are important in determining cell behavior, can influence the outcome of irradiation\(^{11-14}\). For example, transfer of genes for growth factors or growth factor receptors that cause cell proliferation can often achieve radiation resistance. A dominant negative approach antisense, or antibody directed against, for example EGFR, can result in cellular radiosensitization and the level of expression of EGFR by a tumor can determine radiocurability\(^{15-17}\). Radiosensitization often results from transfer of cytokine genes or receptors that slow cell cycle progression or encourage apoptosis.

Radiosensitivity is also related to histological classes. Leith studied the \textit{in vitro} X-ray radiation survival characteristics of 181 cell lines from 12 different classes of exponentially growing human tumor cells (sarcomas, lung cancers, colorectal cancers, medulloblastomas, melanoma, breast cancers, prostate cancers, renal cell cancers, grades III and IV brain tumors, ovarian, head and neck cancers). Radiosensitivities could roughly be divided into two groups; the more radiosensitive group and the more radioresistant group. The intrinsic radiosensitivity of human tumor cells exists among different histological classes of neoplasm. If, however, tumors contained on average 20 percent hypoxic cells, the dose needed for equivalent cell killing increased by about a factor of 2.6 - 2.8. Also, there was no correlation between the rankings of relative radiosensitivities of the various classes of tumor cells at high doses (as in radiosurgery) to the sensitivity at low doses (as in conventional fractionated radiotherapy)\(^{18}\).

3 Radiosensitive Agents in Colorectal Cancer

3.1 Fluorodeoxyuridine

5-Fluorouracil (5-FU) is one of the most widely used chemotherapeutic agents, and is known to be a radiosensitizer. The combination of fluoropyrimidines and radiation has resulted in increased control of colorectal cancer in the clinic. In 1980 the North Central Cancer Treatment Group and the Mayo Clinic compared adjuvant combination chemotherapy and irradiation to radiation alone. Patients undergoing combination chemotherapy and irradiation had an improvement in both disease-free and overall survival rates compared with those undergoing irradiation alone\(^{19}\). Crane compared the outcome from preoperative chemoradiation and from radiation therapy in the treatment of rectal cancer in two large, single-institutional experiences. Multivariate analysis of the patients in these groups showed that the use of concurrent 5-FU with preoperative radiation therapy for T3 and T4 rectal cancer independently increases tumor response and may contribute to increased sphincter preservation in patients with low rectal cancer\(^{20}\). More recently, 5-Fluorouracil has been shown to be a radiosensitizer and acts in part to increase the susceptibility of tumor cells to the damaging effects of radiation\(^{21}\). Combined radiation with 5-FU-based chemotherapy is more efficacious than radiation alone in patients with squamous cell cancer of the anus. Similarly, combined chemoradiation has been shown by Minsky et al. to be more effective than radiation therapy as a neoadjuvant regimen in patients with rectal cancer\(^{22}\). Minsky and colleagues compared two groups of unresectable patients who were nonrandomly treated with combined chemoradiation or radiation alone. In patients undergoing chemoradiation (\(n=20\)), there was a higher com-
plete pathologic response compared with the 11 patients who underwent radiation alone (20% vs. 0%).

The radiosensitization by 5-fluorodeoxyuridine is in part due to alteration of cell kinetics and redistribution of cells throughout the cycle. In laboratory preliminary work showed that 2 h exposures of HT 29 human colon carcinoma cells to relatively low levels of 5-fluorodeoxyuridine resulted in extended thymidylate synthase inhibition after the drug was removed (up to 30 h after treatment with 0.5 microM 5-fluorodeoxyuridine). The low cytotoxicity associated with this treatment simplified efforts to test the effects of extended thymidylate synthase inhibition on radiosensitivity of HT 29 cells. Although thymidylate synthase was completely inhibited at the end of the 2 h exposure, an increase in the radiosensitivity of the cells was not evident until 16 h after the removal of drug. Flow cytometric analysis showed that cells accumulated in early S phase over time, and the increase in radiation sensitivity of the entire population followed the increase of the proportion of cells in early S phase, a relatively radiosensitive phase of the cell cycle. This treatment schedule was compared with 24 h continuous exposure, and was found that the same maximum increase in radiosensitivity was achieved by both treatment strategies. However, more cytotoxicity was associated with continuous exposure.[23]

Adenoviral transduction of the Escherichia coli thymineless growth requirement uracil phosphoribosyltransferase (UPRT) gene induced marked sensitivity in human colon cancer cells to 5-FU. Kayama investigated the efficacy of virally directed UPRT and 5-FU to enhance the radiosensitivity of HT 29 human colon cancer cells. In vitro chemoradio-gene therapy using the UPRT/5-FU/radiation system showed tumor regressive effects even against large HT 29-established subcutaneous tumors in nude mice.[24]

3.2 Protein kinase C-specific inhibitor PKC412

The cellular response to ionizing radiation is governed by the DNA-damage recognition process but is also modulated by cytoplasmic signal transduction cascades that are part of the cellular stress response. Growth-promoting protein kinase C activity antagonizes irradiation-induced cell death, and, therefore, protein kinase C inhibitors might be potent radiosensitizers. The antiproliferative and radiosensitizing effect of the novel N-benzoylated staurosporine analogue PKC412 was tested in vitro against genetically defined p53-wild type (+/+) and p53-deficient (-/-) murine fibrosarcoma cells and in vivo against radiosensitive p53 +/− and murine fibrosarcoma and human colon adenocarcinoma tumor xenograft (SW480, p53-mutated). PKC412 sensitized both p53 +/+ and p53 −/− tumor cells in vitro and in vivo for treatment with ionizing radiation but with a different mechanism of radiosensitization depending on the p53 status. In p53 +/+ , cells combined treatment with PKC412 and ionizing radiation drastically induced apoptotic cell death, whereas no apoptosis induction could be observed in p53-deficient cells in vitro and in histological tumor sections. Combined treatment resulted in an increased G2 cell cycle distribution in p53 −/− cells at PKC412 concentrations that did not alter cell cycle distribution when applied alone. In vivo, a minimal treatment regimen during 4 consecutive days of PKC412 (4 × 100 mg/kg) in combination with ionizing radiation (4 × 3 Gy) exerted a substantial tumor growth delay for both p53-disfunctional tumor xenografts and showed that the clinically relevant protein kinase C inhibitor PKC412 is a promising new radiosensitizer with a potentially broad therapeutic window.[27]

3.3 Survivin

Spontaneous apoptosis has been shown to predict tumor response to radiochemotherapy in rectal cancer in vivo. Recently, a novel member of the inhibitor of apoptosis protein family, designated survivin, was identified. The inverse correlation of survivin-expression with spontaneous and radiation-induced apoptosis suggests that survivin is an important inhibitor of apoptosis in colorectal cancer cell lines. Analysis of survivin mRNA or protein expression may therefore provide predictive information on radio- and chemoresistance of individual colorectal tumors.[25]. Rodel investigated the impact of survivin expression on tumor cell apoptosis in three colorectal cell lines of different intrinsic radiosensitivities. In vitro analysis revealed higher spontaneous and higher radiation-induced apoptosis rates in the radiosensitive line (SW 48), as compared with the more resistant line (SW 480). SW 480 was characterized by a higher spontaneous expression and a pronounced induction of survivin 48 h after irradiation, whereas survivin expression was low when untreated and not increased after irradiation in the most radiosensitive line SW 48.

3.4 Caffeine

Boonkitticharoen investigated the effect of caffeine, the methylated xanthine, in sensitizing the lethal action of ionizing radiation in vitro in human cancer cells. Plateau phase cultures of colon adenocarcinoma, after absorbing doses of 2 Gy, survived at a rate of 56.30 per cent for colon cancer.[26]

3.5 Bromodeoxyuridine and iododeoxyuridine

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Nitric oxidize (NO) is another potential promising radiosensitizer. Multiple studies using NO donors have examined its effects in radiosensitizing tumor cells. Initial studies examining NO donors indicated that NO enhanced the radiosensitivity of hypoxic mammalian cells in vitro[28]. NO’s radiosensitizing property was first demonstrated in 1957 by Howard Flanders in Nature[29]. Flanders interest in NO was based on the fact that like oxygen, NO had a reactive electron making it a free radical. He hypothesized and demonstrated that NO because of this property could effectively substitute for oxygen as an electrophile and sensitize bacteria to radiation under anaerobic conditions. Subsequently others have shown that NO could also radiosensitize normal human cells[28,30,31]. NO was found to be as effective as oxygen in radiosensitizing hypoxic mammalian cells[30]. One group demonstrated that cytokines could induce endogenous NO production and radiosensitize hypoxic breast cancer cells[32].

NO itself also has effect on colorectal cancer. In one large study of colorectal cancers, iNOS activity and protein expression correlated inversely with advanced stage of disease[33]. Pre-malignant colorectal adenomas may have the highest iNOS activity. iNOS overexpression in these polyps is associated with a specific point mutation in the p53 gene, suggesting that NO may function to initiate development of colorectal cancer rather than stimulating cancer progression[34]. Other studies in human colorectal cancer corroborate this inverse relationship between iNOS expression and tumor progression[35]. Lack of iNOS in knock-out mice promotes intestinal tumors further substantiating the role of iNOS in host defense against colorectal cancer[36]. Given these studies, the use of iNOS gene transfer would be a rationale means to improve the impairment in tumor defense mechanisms that utilize NO.

Although NO is a promising radiosensitizer, and NO itself induce apoptosis, the use of NO donors to augment the effects of radiation in vivo has significant limitations, since in vivo administration of these agents results in systemic hypotension and may increase tumor perfusion and oxygenation, potentially promoting tumor growth[37]. Overexpression of iNOS in tumors by localized direct intratumoral injection of the iNOS gene has the potential of minimizing the systemic side effects of NO while maintaining the salutary tumoricidal effects of high output paracrine NO release. We have previously examined the effects of direct intratumoral gene delivery of iNOS combined with both single and multifractionated irradiation on growth of HCT-116 colorectal tumors in nude mice. Adenoviral delivery of HCT-116 tumors significantly (\( P < 0.005 \)) delayed tumor doubling time and growth when combined with single or multifractionated radiation in nude mice. We have previously demonstrated that adenoviral delivery of the iNOS gene enhances radiation-induced apoptosis in colorectal cancer cells[38]. We have also demonstrated that overexpression of the human inducible nitric oxide synthase gene by adenoviral gene delivery radiosensitizes both human colorectal cancer cells and tumors associated with increased apoptosis in nude mice[39]. The mechanism of NO radiosensitization may be that NO increases angiogenesis and then increases oxygen delivery.

Correspondence to:
Zifa Wang
Orient Organ Transplantation Center
Tianjin First Central Hospital
Tianjin 300192, China
Telephone: 01186-22-2362-6560
Fax: 01186-22-2368-2662
Email: wangzl5213@yahoo.com

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