The Radiosensitive Therapy for Colorectal Cancer

Zifa Wang[1,2], Tracy Cook[1], David Blumberg[1]

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, Survivn, 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-α)) 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 *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-Flurouracil 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 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 vivo 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 radioresistant p53 −/−

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 G_2 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[7].

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

Bromodeoxyuridine (BrdU) and iodo-deoxyuridin (IdU) have similar the radiosensitizing effects on colorectal cancer. Miller conducted concurrently to characterize its effects on the shape of the radiation survival curves of cells of two human colon cancer cell lines, HT 29 and HCT 116. The efficiency of radiosensitization by BrdU, expressed as a function of percentage thymidine replacement, was lower when compared to IdU in both cell lines. The major radiosensitizing effect of BrdU was manifest as an increase in the initial slope (alpha), just as observed for IdU. However, with BrdU, in contrast to IdU, an increase in curvature (repairable damage) was also evident. Cells of the more radiosensitive line, HCT 116, showed less sensitization by either BrdU or IdU than cells of the more radioresistant line, HT 29. These results were consistent with the proposed mechanism of radiosensitization being an increase in the single-hit character of low-LET radiation. The radiosensitizing effects of both analogs were largest in the low-dose region of the survival curve[27].

3.6 Nitric oxidize

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 hu-

man 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. AdiNOS treatment of HCT-116 tumors significantly ($P \leqslant 0.005$) 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:wangzf15213@yahoo.com

References

1. Yahanda AM, Chang AE. Colorectal cancer. In Lazar J. Greenfield: Surgery: scientific principles and practice. Lippincott Williams & Wilkins. 2001:1110 – 31.
2. Saha S, Wiese D, Badin J, Beutler T, Nora D, Ganatra BK, Desai D, Kaushal S, Nagaraju M, Arora M, Singh T. Tech-

nical details of sentinel lymph node mapping in colorectal cancer and its impact on staging. Ann Surg Oncol 2000;7:120 – 4.

3. Blumberg D, Ramanathan RK. Treatment of colon and rectal cancer. J Clin Gastroenterol 2002;34:15 – 26.

4. Gerard A, Buyse M, Nordlinger B, Loygue J, Pene F, Kempf P, Bosset JF, Gignoux M, Arnaud JP, Desaive C, et al. Preoperative radiotherapy as adjuvant treatment in rectal cancer, final results of a randomized study of the European Organization for Research and Treatment of Cancer (E-ORTC). Ann Surg 1988;208:606 – 14.

5. Swedish Rectal Cancer Trial. Improved survival with preoperative radiotherapy in resectable rectal cancer. N Engl J Med 1997;336:980 – 7.

6. Minsky BD, Cohen AM, Enker WE, Sigurdson E. Phase I/II trial of peri-operative radiation therapy and coloanal anastomosis in distal invasive resectable rectal cancer. Int J Rad Oncol Biol Phys 1992;23:387 – 92.

7. Zaugg K, Rocha S, Resch H, Hegyi I, Oehler C, Glanzmann C, Fabbro D, Bodis S, Pruschy M. Differential p53-dependent mechanism of radiosensitization *in vitro* and *in vivo* by the protein kinase C-specific inhibitor PKC412. Cancer Res 2001;61:732 – 8.

8. Hoskin PJ, Saunders MI, Dische S. Hypoxic radiosensitizers in radical radiotherapy for patients with bladder carcinoma: hyperbaric oxygen, misonidazole, and accelerated radiotherapy, carbogen, and nicotinamide. Cancer 1999;86:1322 – 8.

9. Rischin D, Peters L, Hicks R, Hughes P, Fisher R, Hart R, Sexton M, D'Costa I, von Roemeling R. Phase I trial of concurrent tirapazamine, cisplatin, and radiotherapy in patients with advanced head and neck cancer. J Clin Oncol 2001;19:535 – 42.

10. Malcolm Alison. Cancer Handbook. New York: Nature Pub. Group. 2002:1360 – 9.

11. Green SL, Giaccia AJ. Tumor hypoxia and the cell cycle: implications for malignant progression and response to therapy. Cancer Journal from Scientific American 1998;4:218 – 23.

12. McBride WH, Dougherty G. J. Radiotherapy for genes that cause cancer. Nature Medicine 1995;1:1215 – 7.

13. Biaglow JE, Cerniglia G, Tuttle S, Bakanauskas V, Stevens C, McKenna G. Effect of oncogene transformation of rat embryo cells on cellular oxygen consumption and glycolysis. Biochemical and Biophysical Research Communications 1997; 235:739 – 42.

14. Chiang CS, Sawyers CL, Mcbride WH. Oncogene expression and cellular radiation resistance: a modulatory role for c-myc. Molecular Diagnosis 1998;3:21 – 8.

15. Reardon DB, Contessa JN, Mikkelsen RB, Valerie K, Amir C, Dent P, Schmidt-Ullrich RK. Dominant negative EGFR-CD533 and inhibition of MAPK modify JNK1 activation and enhance radiation toxicity of human mammary carcinoma cells. Oncogene 1999;18:4756 – 66.

16. Milas L, Mason K, Hunter N, Petersen S, Yamakawa M, Ang K, Mendelsohn J, Fan Z. *In vivo* enhancement of tumor radioresponse by C225 antiepidermal growth factor receptor antibody. Clinical Cancer Research 2000;6:701 – 8.

17. Akimoto T, Hunter NR, Buchmiller L, Mason K, Ang KK, Milas L. Inverse relationship between epidermal growth factor receptor expression and radiocurability of murine carcinomas. Clinical Cancer Research 1999;5:2884 – 90.

18. Leith JT, Cook S, Chougule P, Calabresi P, Wahlberg L, Lindquist C, Epstein M. Intrinsic and extrinsic characteristics of human tumors relevant to radiosurgery: comparative cellular radiosensitivity and hypoxic percentages. Acta Neurochir Suppl (Wien) 1994;62:18 – 27.

19. Krook JE, Moertel CG, Gunderson LL, Wieand HS, Collins RT, Beart RW, Kubista TP, Poon MA, Meyers WC, Mailliard JA. Effective surgical adjuvant therapy for high-risk rectal carcinoma. N Engl J Med 1991;324:709 – 15.

20. Crane CH, Skibber JM, Birnbaum EH, Feig BW, Singh AK, Delcos ME, Lin EH, Fleshman JW, Thames HD, Kodner IJ, Lockett MA, Picus J, Phan T, Chandra A, Janjan NA, Read TE, Myerson RJ. The addition of continuous infusion 5-FU to preoperative radiation therapy increases tumor response, leading to increased sphincter preservation in locally advanced rectal cancer. Int J Radiat Oncol Biol Phys 2003;57:84 – 9.

21. Rotman M, Aziz H. Concomitant continuous infusion chemotherapy and radiation. Cancer 1990;65:823 – 35.

22. Minsky BD, Cohen AM, Kemeny N, Enker WE, Kelsen DP, Reichman B, Saltz L, Sigurdson ER, Frankel J. Enhancement of radiation-induced downstaging of rectal cancer by fluorouracil and high-dose leucovorin chemotherapy. J Clin Oncol 1992;10:79 – 84.

23. Miller EM, Kinsella TJ. Radiosensitization by fluorodeoxyuridine: effects of thymidylate synthase inhibition and cell synchronization. Cancer Res 1992;52:1687 – 94.

24. Koyama F, Fujii H, Mukogawa T, Ueno M, Hamada H, Ishikawa H, Doi S, Nakao T, Matsumoto H, Shimatani H, Takeuchi T, Nakajima Y. Chemo-radio-gene therapy for colorectal cancer cells using Escherichia coli uracil phosphoribosyltransferase gene. Anticancer Res 2003;23:1343 – 8.

25. Rodel C, Haas J, Groth A, Grabenbauer GG, Sauer R, Rodel F. Spontaneous and radiation-induced apoptosis in colorectal carcinoma cells with different intrinsic radiosensitivities: survivin as a radioresistance factor. Int J Radiat Oncol Biol Phys 2003;55:1341 – 7.

26. Boonkitticharoen V, Laohathai K, Puribhat S. Differential radiosensitization of radioresistant human cancer cells by caffeine. J Med Assoc Thai 1993;76:271 – 7.

27. Miller EM, Fowler JF, Kinsella TJ. Linear-quadratic analysis of radiosensitization by halogenated pyrimidines. II. Radiosensitization of human colon cancer cells by bromodeoxyuridine. Radiat Res 1992;131:90 – 7.

28. Mitchell JB, Wink DA, DeGraff W, Gamson J, Keefer LK, Krishna MC. Hypoxic mammalian cell radiosensitization by nitric oxide. Cancer Res 1993;53:5845 – 8.

29. Howard-Flanders P. Effect of nitric oxide on the radiosensitivity of bacteria. Nature 1957;180:1191 – 2.

30. Griffin RJ, Makepeace CM, Hur WJ, Song CW. Radiosensitizing of hypoxic tumor cells *in vitro* by nitric oxide. Int J Radiat Oncol Biol Phys 1996;36:377 – 83.

31. Dewey DL. Effect of oxygen and nitric oxide on the radiosensitivity of human cells in tissue culture. Nature 1960;186:780 – 2.

32. Janssens MY, Van den Berge DL, Verovski VN, Monsaert C, Storme GA. Activation of inducible nitric oxide synthase results in nitric oxide- mediated radiosensitization of hypoxic EMT-6 tumor cells. Cancer Res 1998;58:5646 – 8.

33. Ambs S, Bennett WP, Merriam WG, Ogunfusika MO, Oser SM, Harrington AM, Shields PG, Felley-Bosco E, Hussain SP, Harris CC. Relationship between p53 mutations and inducible nitric oxide synthase expression in human colorectal cancer. J Natl Cancer Inst 1999;91:86 – 8.

34. Ropponen KM, Kellokoski JK, Lipponen PK, Eskelinen MJ, Alanne L, Alhava EM, Kosma VM. Expression of inducible nitric oxide synthase in colorectal cancer and its assocation with prognosis. Scand J Gastroenterol 2000;35:1204 – 11.

35. Lala PK, Orucevic A. Role of nitric oxide in tumor progression: lessons from experimental tumors. Cancer and Metastasis Reviews 1998;17:91 – 106.

36. Scott DJ, Hull MA, Cartwright EJ, Lam WK, Tisbury A,

Poulsom R, Markham AF, Bonifer C, Coletta PL. Lack of inducible nitric oxide synthase promotes intestinal tumorigenesis in the APC(Min/+) mouse. Gastroenterology 2001;121:889 − 99.

37. Van de Casteele M, Hosli M, Sagesser H, Reichen J. Intraportal administration of glyceryl trinitrate or nitroprusside exerts more systemic than intrahepatic effects in anaesthetised cirrhotic rats. J Hepatol 1999;31:300 − 5.

38. Chung P, Cook T, Liu K, Vodovotz Y, Zamora R, Finkelstein S, Billiar T, Blumberg D. Overexpression of the human inducible nitric oxide synthase gene enhances radiation-induced apoptosis in colorectal cancer cells via a caspase-dependent mechanism. Nitric Oxide 2003; 8:119 − 26.

39. Wang Z, Cook T, Alber S, Liu K, Kovesdi I, Watkins SK, Vodovots Y, Billiar TR, Blumberg D. Adenoviral gene transfer of the human inducible nitric oxide synthase gene (iNOS) enhances the radiation response of human colorectal cancer associated with alterations in tumor vascaularity. Cancer Research 2004;64(4):1386 − 95.