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Molecular Basis of Radioresistance

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INTRODUCTION

IONISING RADIATION is known to induce DNA damage and especially double strand breaks (DSB). Subsequent biological responses, in particular repair, cell cycle arrest, and physiological cell death (apoptosis), necessitate recognition of the damage and subsequent mobilisation of a spectrum of proteins. It has been demonstrated that intracellular signalling via phosphorylation pathways govern biological response to radiation exposure [1–9]. We aim to discuss some of the molecular components now under intensive investigation, which are involved in these processes and which determine the genetic basis of radioresistance.

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IRRADIATION AND CELL CYCLE

There is substantial experimental evidence indicating that radioresistant cell lines tend to display longer division delays than their radiosensitive parents [6]. Regulation of cell cycle transition is therefore considered a keystone in ultimate radiation response. The eukaryotic cell cycle has various characteristic checkpoints regulating transition from G0 to G1 (called START), G1 to S and G2 to mitosis. Although radiation is able to induce different cell cycle arrests, most information has been gathered on G1/S and G2/M arrests which affect DNA damage repair [6].

One important factor in postirradiation G1 arrest appears to be p53 [4, 6]. The protein undergoes post-translational stabilisation after irradiation (no increase of mRNA). The p53 protein binds to a regulatory sequence controlling the expression of different genes activating muscle creatinine kinase, growth arrest and DNA damage-inducible GADD45 and GADD153; murine double minute *mdm-2* which plays a role in tp53 regulation; and of

particular importance WAF1/CIP1, a potent inhibitor of cdks and hence at the basis of a regulatory loop for p53; suppressing c-fos, c-jun, β-actin, hsp-70 and IL-6. Some of these gene products, for example, GADD45, are induced rapidly and transiently after irradiation and play a central role in the p53-dependent G1 delay. Patients with ataxia telangectasia are deficient in the normal induction of GADD45 mRNA and abnormal in expression of p53 protein, which explains their high radiosensitivity [1, 4, 6].

Commitment to cycle (G0 to G1 transition) requires interaction of $p34^{cdc2}$ and the G1 cyclins in order to progress to S phase. $p34^{cdc2}$ is also a key protein in the G2/M transition. Linked to cyclin-B, it constitutes mitosis promoting factor (MPF). In its activated form (dephosphorylated on residues 14 and 15 by the action of a phosphatase cdc25), cdc2 promotes entry into mitosis by phosphorylating histones (H1) and nuclear lamins which results in chromosome condensation and dissolution of the nuclear envelope [6]. Irradiation induces a dose-dependent down-regulation of cyclin-B1 mRNA and blocks the cells at the G2/M transition. Caffeine, which is known to counter the cell cycle arrests, is also able to accelerate the kinetics of reappearance of cyclin-B1, increasing the radiosensitivity of the cells by shortening the G2 delay [2].

The radiation-induced G2 delay must be triggered by activation of a tyrosine kinase in order to phosphorylate $p34^{cdc2}$ and block the cells by inactivation of MPF [6]. The exact tyrosine kinase activated by irradiation is currently unknown. Irradiation also induces elongation factor EF-1δ, which precedes G2 arrest. This protein is a subunit of EF-1, a complex that participates in the elongation phase of mRNA translation, and is a potential substrate of $p34^{cdc2}$.

IRRADIATION AND APOPTOSIS

Apoptosis occurs both in normal tissues and in tumours irradiated *in vivo* [5]. The extent of apoptosis varies according to the tumour and is related to its radiocurability. This active process is modulated by various factors from different pathways: these include the roles of Ca^{2+}, mRNA and protein synthesis, signal transduction pathways especially protein kinase C (PKC) as a down regulator and protein kinase A (PKA) as a stimulator, and finally polyamines. Knowledge of these pathways should allow therapeutic manipulations aimed at increasing the radiation response [1, 5].

Cyclins such as cyclin A, and other proteins, such as c-myc, p53, cdc-2 and PCNA have been shown to rise dramatically during apoptosis [1, 7]. Increase of p53 and c-myc (both cell cycle regulating proteins) has been observed in the blebbing nuclei of cells undergoing apoptosis. It is interesting to note that overexpression of wildtype (wt) p53 can result in growth arrest or can elicit apoptosis [7]. The type of response depends on the intracellular context, i.e. the presence or absence of growth factors such as IL-3 (known to increase the radiation resistance in haematopoietic cells).

IRRADIATION AND GROWTH FACTORS MEDIATED RESPONSE

Receptors activated by autocrine (receptor and ligand on the same cell), paracrine (on different cells) or juxtacrine (on adjacent cells) ligands, secondary to environmental factors such as cytokines and growth factors, ionic flux or nucleus DNA damage, induce a cascade of enzyme activation [3, 9]. These enzymes, kinases and phosphatases (secondary messengers), respectively phosphorylate or dephosphorylate target proteins (tyrosine kinase, PKC, mitogen activated protein kinase MAPK), and ultimately alter nuclear gene expression with the appearance of early response gene transcripts (the rapid response does not require *de novo* protein synthesis) such as c-jun, c-fos, EGR1, GADD45, NFκB and finally the late response gene transcripts such as TNF, TGF-β, IL1 and β-FGF. It is currently unknown if there are specific radiation-response elements (RRE).

Growth factors or cytokines induced by radiation exposure include tumour necrosis factor (TNF-α), platelet derived growth factor β-chain (PDGF-β), basic fibroblast growth factor (b-FGF), interleukin-1 (IL-1), and transforming growth factor β (TGF-β) [3, 9]. Some of those growth factors have different actions according to the target cell. TNF-α for example, is able to protect bone marrow progenitor cells against radiation while it sensitises tumour cells or is directly cytotoxic.

Radiation-induced transcriptional activation of TNF-α can, through activation of a phospholipid signal pathway (S-Mase pathway), result in induction of early response genes related to apoptosis such as *NFκB*. However, radiation can trigger PKC (protein kinase C: down regulator of apoptosis) and S-Mase. Repair of damage or apoptosis will ultimately depend on the relative balance between S-Mase and PKC pathways. This balance will depend upon the relative amounts of cell surface receptors (p55 for induction of early response genes and p70 mediating cell kill) between different types of target cells. The TNF effect on radiosensitivity can also be explained by induction of the MnSOD gene (manganese superoxide dismutase). MnSOD is a free radical scavenging protein and plays a key role in cellular oxydoreduction mechanisms.

NFκB is an early response gene induced by hypoxia or reactive oxygen intermediates. These agents trigger membrane associated kinases (src, ras and raf), resulting in the tyrosine kinase activity (phosphorylation), required for activation of *NFκB* via cleavage of IκBα. A residual p50/p65 heterodimer is formed, initially located in the cytoplasm but translocated to the nucleus where it binds with high affinity to a κB regulatory sequence. Many stress-inducible genes and genes coding for cytokines increase their expression upon activation by *NFκB* and influence apoptosis.

Interleukin-1 (IL-1) protects bone marrow cells by promoting growth and proliferation of early progenitor cells. Like TNF, IL-1 activates free radical scavengers and various growth factors. Basic fibroblast growth factor (β-FGF) appears after irradiation and is thought to be involved in potentially lethal damage repair. β-FGF affects the width of the shoulder of the dose response and hence the capacity to repair damage, whereas the D0 remains unchanged (linear component related to unrepaired DNA DSB). Finally, β-FGF mediates inhibition of radiation induced apoptosis through activation of PKC. Interphase apoptotic cell death may therefore prevail over postmitotic cell death in the shoulder region of the dose response.

CONCLUSIONS

Post-irradiation DNA damage or other clastogens will initiate cell cycle delay, extending repair of DNA damage, or will activate nuclear blebbing, endonuclease activity and chromosomal condensation, features characteristic of apoptosis. Exact knowledge of the molecular mechanisms behind the response to ionising radiation is essential for the development of biologically based treatment schedules with a higher therapeutic index.

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News

New Appointment

Dr Alberto Costa, Executive Director of the European School of Oncology, has been elected to the position of Secretary of the Federation of European Cancer Societies. Dr Costa will take up office immediately after ECCO 8 in November this year. He will serve for a period of 2 years and replaces Professor Emmanuel van der Schueren.

EORTC New Drug Development Office—Vacancy

The EORTC New Drug Development Office (NDDO) is the executive office of the EORTC research division and as such is responsible for the coordination of a comprehensive anticancer drug development programme in Europe. The office covers all the essential preclinical and clinical development stages such as screening, formulation, toxicology as well as Phase I and early Phase II clinical trials. Studies are performed in collaboration with EORTC-cooperative groups and the international pharmaceutical industry. The EORTC-NDDO is located at the Free University Hospital in Amsterdam, The Netherlands. The EORTC-NDDO at present has 17 employees; the director is supported by a multi-disciplinary staff consisting of a medical oncologist, pharmacist and biologist and including advisors in automisation, finances and biostatics.

The EORTC invites applicants for the position of director. The applicant should be an M.D. with extensive experience in medical oncology, preferably holding a PhD degree. Expertise in the clinical pharmacology of cytoxic agents, as well as in the coordination of cancer research programmes is required. Previous administrative experience, and leadership/multilingual skills are necessary. Applicants should send a letter of intent, CV and names of three referees within 2 weeks to the President of the Foundation NDDO: MR R.J.Vless, c/o Advocatenkantoor Ekelmans-Den Hollander, P.O. Box 545, 1000 AM Amsterdam, The Netherlands; Tel: 31-20-553-3600. For more information, please contact the above address. Applicants are informed that there is an internal candidate.

The Division of Colon and Rectal Surgery of the University of Minnesota Medical School

The 2nd Annual Conference, "Molecular Biology of Colorectal Cancer", will be held at the University of Minnesota, Minneapolis campus, on 12 September 1995. The fee is $125 and the accreditation is 7 hours, Category 1-AMA. For further information, please contact Continuing Medical Education, University of Minnesota, Radisson Hotel Metrodome, Suite 107, 615 Washington Avenue S.E., Minneapolis, Minnesota 55414, U.S.A.; or call (612) 626-7600; toll free 1-800-776-8636; Fax: (612) 626-7766.

Second University of Chicago Symposium. Concomitant Chemoradiotherapy for Solid Tumours. Symposium Director: Everett E. Vokes M.D.

This will be held between 28 and 29 September 1995 at the Ritz Carlton Hotel, Chicago, Illinois, U.S.A. For further information, please contact The Center for Continuing Medical Education, The University of Chicago, 6019 South Kimbark, Box 129, Chicago, Illinois 60637, U.S.A.; Tel: (312) 702-1056; Fax: (312) 702-1736.

Chemotherapy Foundation Symposium XIII, Innovative Cancer Chemotherapy for Tomorrow

This conference will be held between 1 and 3 November 1995 at the Crowne Plaza in New York and is sponsored by the Division of Neoplastic Diseases and the Post-Graduate School of the Mount Sinai School of Medicine with Ezra M. Greenspan M.D. as Chairman. For a programme and further information, please contact Jaclyn Silverman, Division of Neoplastic Diseases, Box 1178, Mount Sinai School of Medicine, One Gustave Levy Place, New York, New York 10029, U.S.A.; Tel: (212) 241-6772; Fax: (212) 996-5787.

The International Society For Haematology and Graft Engineering (ISHAGE)

The 2nd International Meeting of this Society is being held between 21 and 23 June 1995 in Vancouver, Canada. In three action packed days, you will attend Plenary and Scientific Update Forums featuring leaders in the field, including keynote speaker, Dr E. Donnall Thomas. In addition, standards, accreditation and regulatory issues that affect your laboratory and clinical practice will be discussed, and technical workshops at both a basic and advanced level will be held. For further information, please contact Stephanie Hudson, Conference Organizer, Terry Fox Laboratory, 601 West 10th Avenue, Vancouver, B.C., Canada, V5Z 1L3; Tel: (604) 875-4961; Fax: (604) 875-5053.