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Eur J Cancer, Vol. 27, No. 4, pp. 405–407, 1991.
Printed in Great Britain

0277-5379/91 $3.00 + 0.00
Pergamon Press plc

Possible Applications of Biotechnology to Radiotherapy

INTRODUCTION

MUCH OF the research emphasis in radiation oncology in the USA and Europe has focussed on improving the technical precision of radiotherapy delivery, understanding the effects of fractionation on tumours and normal tissues and strategies to overcome hypoxia as a therapeutic limitation. Detailed mathematical modelling of *in vitro* human tumour cell survival curves and *in vivo* normal tissue effects has been undertaken by some radiobiologists and radiotherapists without sufficient emphasis on potentially important advances in the molecular aspects of radiotherapy. The evolution of this relatively narrow focus is paradoxical since in the previous 20 years radiotherapists/radiobiologists have made seminal discoveries in biology, such as the application of a colony forming assay to study lethality induced by cytotoxins in mammalian cells, the cell cycle specificity of radiation killing, and the repair of sublethal and potentially lethal X-ray damage, to name just a few accomplishments. Thus it seems appropriate to look forward toward the potential applications of molecular biology to radiotherapy.

MOLECULAR BIOLOGY OF RADIORESISTANCE

A fundamental understanding of the molecular basis of radioresistance may be important in identifying genetic and biochemical targets to enhance the lethal effects of X-rays on tumours or reversing these effects in normal tissues. Studies in humans of DNA repair deficient syndromes such as ataxia telangiectasia and xeroderma pigmentosum strongly suggest that the response to physical agents in human cells has a genetic basis [1]. There is an interest in cloning genes that repair DNA damage; however, to date most of the mammalian repair genes cloned and characterised repair alkylating agent or ultraviolet light induced DNA damage [1–3]. Genes likely to be involved in the repair of lethal X-ray damage will probably encode for products that repair DNA double strand breaks. Thompson has pointed out that regulation of radiation repair genes may only explain radioresistance (greater than wild type survival), if the gene products are rate limiting in the biochemical steps of DNA repair [3]. Nonetheless identification of genes important in the repair of lethal X-ray damage presents exciting possibilities such as prediction of radiocurability or potentially manipulation of the radiation response in the clinic. Activation of the oncogenes *ras*, c-*raf* and c-*mos* have been shown in a variety of laboratory settings to confer radioresistance on cells [4–7]. Recently, this effect has been suggested to be the result of abnormal intracellular signal transduction with cell cycle alteration by aberrant oncogene products [8]. Hartwell and Weinert suggest that genetic control mechanisms in the cell cycle called "checkpoints" result in a cessation of cell cycle progression following X-ray and alkylating agent treatment in cells and that this growth arrest allows for repair of mutagen induced DNA damage [9]. These mutagen induced checkpoints may also be responsible for normal distribution of chromosomes or other organelles in a viable mitosis following DNA damage. Thus, combination of checkpoints followed by DNA repair (reminiscent of the potentially lethal damage repair) may be important in the survival of tumours and normal tissues following ionising radiation or other cytotoxic agents. Yeast genetics might be a good starting point to identify genes that control the cell cycle and subsequent survival after X-rays and thus have potential relevance for radiotherapy. The identification of the human homologs of the highly conserved *cdc*-2 and *cdc*-25 genes provide ample evidence of the feasibility and potential importance of this approach [10,11].

GENETIC SUSCEPTIBILITY TO CANCER

Families with a genetic predisposition to malignant tumours may provide important clues as to the genetic events involved in the various stages of carcinogenesis. Hereditary mutations at tumour suppressor loci may confer a highly penetrant predisposition to cancer. For example, patients with hereditary retinoblastoma have a high incidence of radiation induced osteosarcomas as well as spontaneous osteosarcomas distant from the irradiated site. This clinical observation suggested that inactivation of the retinoblastoma tumour suppressor gene may be involved in the aetiology of some osteo- and soft-tissue sarcomas [12, 13]. Recent findings suggest that inheritance of variant p53 alleles is associated with the Li–Fraumeni syndrome. This is a rare familial cancer syndrome characterised by a predisposition to breast carcinoma, soft tissue sarcomas, brain tumours, leukaemia and adrenal cortical carcinoma. Other possible components of the Li–Fraumeni syndrome are: melanoma, gonadal germ cell tumours and carcinoma of the lungs, pancreas and

Received 16 Jan. 1991; accepted 22 Jan. 1991.

prostate [14, 15]. In a large scale of screening for germinal mutations of the p53 gene in sporadic and familial bone and soft tissue sarcomas, our group in collaboration with colleagues at the Massachusetts Eye and Ear Infirmary found both hereditary and new germinal mutations of the p53 gene. Not all of the patients with germinal p53 mutations were Li–Fraumeni like in their history and some patients suffered radiation induced sarcomas. This finding adds to the data that genetic susceptibility to some human cancers may be due to an inherited mutation in at least one allele of a tumour suppressor gene and suggests some patients with germinal mutations in tumour suppressor genes are susceptible to radiation (or chemotherapy) induced second tumours. As cancer therapy improves, the problem of second neoplasms will increase. For example, 10–15% of patients with Hodgkin's disease develop second malignant tumours 10–15 years following radiotherapy. Therefore, it is important to identify patients who are at risk for second tumours so that alternative therapies and/or chemopreventive measures might be undertaken. No other group of physicians has followed such a large cohort of patients treated with at least one mutagen (ionising radiation) than radiation oncologists. Therefore it is appropriate for radiotherapists to identify patients at risk for radiation induced or spontaneous second malignancies and counsel patients and families appropriately. As well, radiotherapists should become actively involved in chemoprevention.

GENE MANIPULATION AND RADIOTHERAPY

Our group, in collaboration with colleagues at the University of Chicago and Harvard Medical School, found the transcription factors c-JUN and EGR-1 induced by ionising radiation [16, 17]. These factors are induced at the transcriptional level, are independent of protein synthesis and at least partially dependent on activation of protein kinase C. These data suggest that phosphorylation of proteins initiate transcription of c-JUN and EGR-1 by binding to radiation responsive DNA sequences in the promoter region of these genes. It may be possible to regulate transcription of a variety of genes by placing radiation responsive DNA sequences in front of other genetic constructs which might amplify a radiation induced signal. For example, a genetic construct with the VP16 sequences that code for a known powerful *trans* activating protein of herpes virus attached to the DNA coding sequence derived from the DNA-binding domain of the *lac* repressor placed downstream of DNA sequences that bind radiation inducible proteins might be effective in amplifying a radiation induced signal. This construct could be cotransfected with a plasmid containing multiple DNA binding sites for the *lac* repressor (protein) cloned upstream of a gene which when activated alters the phenotypic response of cells to radiation [tumour necrosis factor (TNF), fibroblast growth factor (FGF), or toxins such as ricin] (R. R. W. *et al.*, patent applied for and refs 18 and 19). There are a variety of problems associated with this approach including insertion of the construct into cells and appropriate targeting to tumours (or normal tissues). It is noteworthy however that TNF has been placed in lymphokine activated killer (LAK) cells by Rosenberg's group and thus a certain amount of targeting is already possible in some tumours. Also pertinent haematopoietic growth factors and cytokines could be placed in haematopoietic stromal cells and subsequently induced during radiotherapy. If this technology develops sufficiently, radiotherapists would be involved in the management of systemic disease since induction of genes could be targeted wherever the radiation was delivered. The Memorial group has shown that radiation induction of platelet-derived growth factor (PDGF) and FGF may be associated with the proliferation of vascular smooth muscle and endothelial cells and suggest these cytokines may contribute to the late effects of radiotherapy [19]. Thus, genetic reversal of cytokine secretion might increase radiation tolerance of late effects in some normal tissues. Another arena where radiotherapists have entered the treatment of systemic disease is through use of radiolabelled monoclonal antibodies. The technology for novel immunological techniques for tumour targeting is emerging. Future improvements in radiochemistry and dosimetry may make radioimmunotherapy part of the future treatment of neoplastic disease.

In summary, radiation oncology should expand its intellectual infrastructure with an eye towards the application of biotechnology to broaden the radiation oncologists clinical horizon. Radiotherapy ought to develop an intellectual diversity and rigour that will allow the specialty to remain at the center of cancer management.

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Acknowledgements—Grateful appreciation to Dr Samuel Hellman and Dr Vikas Sukhatme for their contribution to this editorial. Partially supported by NIH grants CA 42596 and CA 41068.

Eur J Cancer, Vol. 27, No. 4, pp. 407–408, 1991.
Printed in Great Britain

0277–5379/91 $3.00 + 0.00

Infections in Cancer Patients: Differences between Developed and Less Developed Countries?

IN ORDER TO address the question of whether there are differences in infections among patients with cancer in developing compared with developed countries (see the study by A. Awidi, in this issue, pp. 423–426) it is necessary, it seems to me, to first address the key criteria that predispose to infection. I have always found it useful to divide infected patients based upon a fairly limited number of conditions that predispose to different types of infections, namely, granulocytopenia, cellular immune deficiency, humoral immune deficiency, obstructive phenomenon, and procedure related (indwelling intravascular catheter, urinary catheter, respirator, etc.). The types of infections with regard to both site and especially with regard to organism are quite different in each of these settings. The seriousness of the infection and, indeed, the mortality is also different among these settings [1–3].

GRANULOCYTOPENIA

Patients with granulocytopenia tend to have infections caused by aerobic gram-negative bacilli, especially *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*, the gram positive cocci, *Staphylococcus aureus*, *Staph. epidermidis*, and various streptococci, and, among patients further predisposed by broad spectrum antibiotics, fungal infections caused by *Candida albicans* and *C. tropicalis*, and *Aspergillus flavus* and *A. fumigatus*. Of course, other organisms do cause infection, but the ones noted cause the great majority of infections directly related to granulocytopenia. Further, more than half of these infections are caused by strains of these organisms that have been acquired in the hospital setting, thus accounting for institutional patterns of antimicrobial resistance among infections in granulocytopenic patients [4]. In that regard, it is useful to recall that not only therapeutic agents but also the use of prophylactic antibiotics, whether in an inpatient or outpatient setting, may lead to the acquisition or the emergence of resistance patterns (e.g. patients on cotrimoxazole may become colonised with *E. coli* resistant not only to cotrimoxazole but also the broad spectrum antipseudomonal penicillins and some of the cephalosporins) [5].

The patient who is profoundly granulocytopenic (defined as < 100 polymorphonuclear neutrophils per μl) is more likely to have infection proceed to bacteraemia which confers a more grave prognosis. Further, those patients with profound and persistent granulocytopenia who develop a gram negative rod bacteraemia are well recognised to have a much more adverse prognosis with regard to mortality than does the patient who has return of circulating granulocytes over the next few days [6].

What differences might one expect to find among granulocytopenic patients in a developing compared with a developed country? I would assume that the only major differences would relate principally to the acquisition of organisms. If food, water or air are more likely colonised or contaminated in one setting than the other, then acquisition rates might be higher and, hence, infection rates higher. For example it is well known that if *Ps. aeruginosa* colonises a patient who then becomes profoundly granulocytopenic, the patient almost invariably will proceed to a *Ps. aeruginosa* bacteraemia. Therefore, if the patient is in a setting where, because of water handling or food handling practices (ice machines, tap aerators, ingestion of foods commonly colonised by *Ps. aeruginosa*), then one would expect to see more acquisition and, hence, more infection by *Ps. aeruginosas* [7]. The other major factor would be the general approach to the use of antibiotics in an individual country or area. If, for example, cotrimoxazole was an over-the-counter agent available for any number of minor infectious ailments, then one might expect to find in the population at large an increased number of resistant organisms. This would then have a profound impact on the choice of antibiotics for use in empiric therapy.

INFECTIONS IN CELLULAR IMMUNE DEFICIENCY

Some cancers, such as Hodgkin's disease, have an associated cellular immune deficit. Radiation therapy and some forms of cancer chemotherapy along with corticosteroids depress the cellular immune mechanism and, of course, the acquired immunodeficiency syndrome is inherently a disease of cellular immune loss. The infections that occur in these patients are frequently those caused by obligate intracellular parasites that have the capacity to remain latent for many years [8]. Among the bacteria are *Mycobacteria*, *Nocardia*, *Salmonella* and *Listeria* spp.; among yeasts and fungi are *Cryptococcus*, *Histoplasma*, and *Coccidioides*; among the viruses are herpes simplex, varicella zoster, and cytomegalovirus; other organisms include *Strongyloides* and *Pneumocystis carinii*. Infection in these patients is then a conse-

Received 10 Jan. 1991; accepted 11 Jan. 1991.