

# Radiation Oncology and Molecular Biology - The Frontiers Ahead

DIGANTA HAZARIKA\*, TEJINDER KATARIA\*\*

*Departments of Pathology\* & Radiation Oncology\*\*, Rajiv Gandhi Cancer Institute and Research Centre, Rohini, New Delhi, India*

**Abstract:** Many randomized trials conducted over the last few decades show that combinations of radiation and chemotherapy given concurrently decrease the mortality rate of patients with locally advanced cancers of the head and neck, uterine cervix etc. However, as longer follow up data have become available it has become evident that the survival benefit has been achieved at the expense of increased toxicity and also that a substantial percentage of patients die of their index cancer progression. The identification of molecular prognostic markers that can predict the pattern of relapse and serve as a target for intervention to selectively sensitize tumours to radiation may help overcome this situation.

**Key Words :** *Signal transduction, epidermal growth factor, farnesyl transferase, Tyrosine kinase.*

## Introduction

Radiation biology is dedicated to understand the effect of radiation on living beings. To many, the effects of radiation on living organisms are considered paradoxical; radiation is known to cause cancer, yet as administered in clinical radiotherapy, radiation represents the major anticancer modality in terms of successful tumour care and patient survival. Studies on the physical, biological and chemical changes which follow the interaction of radiation with living matter are of fundamental importance in understanding how radiation can be used to investigate normal and aberrant cell structure and function.

**Basic Concept :** Ionizing radiation are of two types-particle and electromagnetic. They cause ejection of electrons with release of energy. Electro-magnetic radiation are usually X-rays and gamma rays. Particle radiation are alpha, beta, proton, neutron and mesons. With a standard dose of radiation, its radiological effect depends on the type of radiation and type of tissue being irradiated. For example bone marrow and epithelium are highly sensitive where as brain is relatively resistant.

Radiation causes interruption of cell replication by causing damage to DNA, proteins and cell water. DNA damage causes single - or double - strands breaks, base alterations and cross linking of molecules. Proteins are denatured, cellular water undergoes radiolysis to make active peroxides, free hydroxyl radicals, electrons, hydrogen atoms and hydrogen peroxide molecules. Free radicals are atoms or molecules with at least one unpaired electron. 70-80% of the effect of radiation on cells is due to production of free radicals. The net result is necrosis and apoptosis with morphological changes like cellular swelling, cytoplasmic vacuolation, altered chromatin pattern, and nuclear vacuolation. The cells with a high mitotic rate are more sensitive than those with a low mitotic rate. Likewise cells with G2 and M cells are more sensitive to radiation than those cells in cycle G1 and G0. Cells are more resistant in the late S phase than other phases of the cell cycle. The cells with high oxygen content are more sensitive than those with low oxygen content. Poorly differentiated or anaplastic cells are more sensitive than well-differentiated tumour cells<sup>1</sup>.

With the development of megavoltage treatment and computerized treatment planning, the quality and precision of radiation oncology has improved phenominally. It has contributed to better local

control for some cancers; however failed to control micrometastasis beyond the radiation treatment field, even with combination systemic treatment.

The discoveries that cancers result from genetic changes such as the activation of an oncogene or the loss of a tumour suppressor gene has offered new opportunities for targeting the tumour. Specifically, the finding that over expression of growth factor signal transduction pathways can drive uncontrolled tumour cell growth presents the opportunity to target specific genetic alterations that produced and support the growth of the cancer. The discovery has led to generation of antibodies and small molecules aimed at inhibiting aberrant growth factor receptor activation. This is a review of some of the basic biology and very early clinical results of combining these new "molecularly targeted" therapies with radiation.

## Signal Transduction Pathways

The molecules responsible for transducing signals from receptors to downstream targets have been the subject of intensive investigation in recent years. Among the most well-studied signal transduction pathways are the mitogen-activated protein kinase pathway (MAP kinase) acting mainly at the genetic level in the nucleus; the phosphatidylinositol 3'-kinase pathway (PI3-kinase), the phospholipase 3-gamma pathway active mainly along the cell surface, and the Jun kinase/p38 MAP kinase pathways<sup>2</sup>. Each of these pathways consists of a number of molecules, each of which is responsible for moving the signal from the molecule directly upstream to the one immediately downstream. In most cases, signals are propagated by progressive phosphorylation and dephosphorylation events, but other molecular methods can be operative as well. In addition, docking and scaffolding molecules are involved in placing signals in their appropriate subcellular locations.

There are three broad types of receivers that play important roles in mediating the mitogenic response to growth factors and matrix molecules (a) nucleus (b) cell-surface (c) mitochondria. Theoretically the monoclonal antibodies or small molecule inhibitors of growth factor receptors could have three distinct effects on the cancer cells expressing the target. These are (i) cytostatic activity (ii) cytotoxic activity (iii) potential radiation sensitizers.

There is a mechanistic basis for the ability of these compounds to sensitize cells to radiation because it has been shown that oxidative

stress resulting from radiation exposure can activate signaling pathways important for cell-survival<sup>3</sup>. Thus blockage of these radiation-activated signaling pathways can be expected to sensitize cells to radiation. Radiation is given as a multi fractionated treatment; the expected benefit of the presence of a signal targeting agent would be to block the proliferation of cells that survive each fraction of radiation; also tumour cell-specific cytostatic effects would prevent accelerated repopulation occurring late in the course of the treatment of aggressive tumours. The direct cytotoxic effects of the antibody molecule could have a synergistic effect on the potential gain due to radiation. Hence it is possible that future clinical trials are able to show a positive outcome of receptor targeted therapies along with radiation in tumours expressing, activated targets which act as a primary driving force for their malignant potential.

## Epidermal Growth Factor Receptors and Radiation Response

The epidermal growth factor receptor (EGFR) represents the first member of the Erb B family of receptors. These receptors are transmembrane proteins from the family of type I receptor tyrosine kinases. On ligand binding, it initiates transduction signals that regulate cell division, proliferation, differentiation and death, all of which play an important role in cellular transformation and tumour response to therapy. In the clinical setting the Erb B<sub>1</sub> (EGFR) and Erb B<sub>2</sub> (HER2/neu) receptor are the most well-recognised and understood to date. Trastuzumab (Herceptin) has gained widespread use in the treatment of Erb B<sub>2</sub> over expressing breast cancer patients. The new Erb B<sub>1</sub> inhibitory agents are postulated to provide a clinical benefit to a broad spectrum of patients with epithelial malignancies expressing EGFR<sup>4</sup>. The interaction of EGFR inhibitory agents combined with radiation shows a highly favourable interaction profile in the preclinical studies. The EGFR inhibitory agents are often broadly classified as (a) large molecules including monoclonal antibodies, bi specific antibodies or immunotoxin conjugates. (b) small molecules most commonly include receptor tyrosine kinase inhibitors that are selective for the EGFR e.g. quina zolines.

A number of complementary mechanisms have been identified that may account for the favourable interaction profile observed regarding EGFR inhibitors combined with ionizing radiation. In general, signaling through the EGFR pathway is stimulatory to the cell cycle machinery that controls cells proliferation. Therefore, EGFR inhibitors provide an antiproliferative influence that has been studied in some detail from a mechanistic standpoint. The EGFR inhibitory agents primarily induce G1 cell cycle arrest. Epidermal growth factor receptor signal blockade precipitates a decrease in the activity of cyclin-dependent kinase (CDK2) via an increase in the expression of selected cyclin-dependent kinase inhibitors, such as p27, thereby preventing the transition of cells into S phase<sup>5</sup>. Radiation, on the other hand, most commonly induces G2 cell cycle arrest, which has been well documented in a number of model systems. In general terms, this radiation-induced G2 cell cycle arrest is thought to afford the opportunity for partial or complete repair of radiation damage before resumption of cell cycling. The fact that EGFR inhibitors induce G1 cell cycle arrest, whereas radiation induces a G2 cell cycle arrest, which may itself represent a key mechanistic contributor to the observed success of this combination therapy.

Epidermal growth factor receptor inhibitory agents have also been found to enhance radiation-induced apoptosis and to inhibit radiation-induced damage repair. These effects have been well established in human squamous cell carcinomas treated with the anti-EGFR monoclonal antibody IMC-C225 (Erbbitux, cetuximab: ImClone Systems Incorporated, New York, NY, and Bristol-Myers Squibb Company, Princeton, NJ)<sup>6</sup>. Similar results have recently been confirmed in this same squamous cell carcinoma model system using the EGFR tyrosine kinase inhibitor, ZD1839. A similar pattern of increased radiation effect has been documented in studies examining ErbB2 inhibition. Specifically, enhanced radiation response and diminished DNA repair capacity is observed in human MCF-7/HER2 breast cancer cells following monoclonal antibody blockade of the ErbB2 receptor. Quite distinct from the cell cycle checkpoint modulation described previously, the influence of EGFR blockade on apoptosis and damage repair may represent key contributors to the synergy observed with EGFR inhibition plus radiation in several model systems. It appears that downregulation of selected mitogenic signal transduction pathways can profoundly inhibit cellular recovery processes following radiation damage.

Finally, there is compelling preclinical data regarding the capacity of EGFR inhibition to modulate the processes of tumour invasion and angiogenesis. More specifically, downregulation of EGFR signaling can inhibit tumour angiogenesis via transcriptional downregulation of vascular endothelial growth factor mRNA and resultant protein expression. Several *in vitro* and *in vivo* model systems confirm these effects and may explain why the *in vivo* impact of EGFR inhibitory agents has often proved more potent than that observed in simple cell culture systems.

## Farnesyl transferase inhibitors and radiation sensitization

Activation of Ras, by mutation, overexpression, or by signaling through tyrosine kinase receptors, is associated with radioresistance. Thus, therapies that inhibit Ras function could be an effective means to radiosensitize selected types of solid tumours. Inhibition of Ras prenylation using a variety of farnesyltransferase inhibitors resulted in radiosensitization of tumour cells that expressed activated Ras, both *in vitro* and in xenograft models. Farnesyltransferase inhibitor treatment could also inhibit tumour regrowth following irradiation of mice bearing T24 tumour xenografts that express activated Ras. In a phase I trial of the farnesyl transferase inhibitor L-778-123 and radiotherapy in patients with locally advanced head and neck cancer and non-small cell lung cancer, a high response rate was observed coupled with a mild toxicity profile<sup>7</sup>. Additional clinical trials should shed light on the potential of this and other farnesyl transferase inhibitors to serve as radio-sensitizers and may identify molecular markers that could predict a response to these agents.

## Benzotriazine di-N-oxide and hypoxia

Serendipity played a role in the discovery of a novel hypoxic cell toxin-benzotriazine di-N-oxide, known as TPZ<sup>8</sup>. The known fact that hypoxic cells were resistant to killing by X-ray is exploited for therapeutic benefits thus turning hypoxia from problem to advantage. It acts like a bioreductive drug. It is found that when TPZ is given in combination with radiation for head and neck cancers, TPZ potentiates cell kill by fractionated radiation<sup>9</sup>. Other approaches are being investigated for targeting hypoxia induced

proteins such as HIF-1 or using hypoxia to obtain tumour specific gene expression for gene therapy.

## Tyrosine Kinase inhibitors as Radiation Sensitizers

The discovery of highly selective and potent compounds called the 4-anilinoquinazolines has led to the development of small molecule tyrosine kinase inhibitors as potential anti cancer agents. These agents inhibit essential cellular pathways in growth factor expression and can be administered as an oral formulation. Some of these agents, such as ZD 1839 and OSI-774, tend to bind in vitro only to the epidermal growth factor receptor tyrosine kinase while others, such as CI-1033, bind to multiple members of the ErbB family. The first clinical compounds that were developed such as ZD 1839 were reversible inhibitors. Recently developed irreversible compounds may be found to be more effective at producing long-term suppression.

These molecules have the ability to block cell growth, decrease the clonogenic potential of cells after chronic exposure, down regulate specific genes important for neoplastic potential and to sensitize cells to the lethal effects of ionizing radiation. In vitro studies have shown that combining multifraction radiation exposures (15Gy) with chronic administration of CI-1033, (an irreversible inhibitor of Erb B Kinases) can decrease the number of clonogens in a population of breast cancer cells by nearly a factor of 100 compared to that achieved with radiation alone<sup>10</sup>. Preliminary in vivo studies have shown that the combination of fractionated radiation and CI-1033 can produce a significant growth delay beyond that which occurs from either treatment alone. This occurs even when the cells have only moderate (i.e. clinically relevant) over expression of EGFR. Histopathologic evaluation of tumours treated with radiation and CI-1033 show central tumour and vascular necrosis, suggesting that the combination effect may depend on anti-angiogenic or other cytokine mediated factors.

## Conclusion

The advances in molecular biology and the insight into the mechanisms of neoplastic transformation, progression and its response to radiation therapy is just beginning. A new frontier is being opened up, the vast vista of this knowledge is still in infancy and the next few years will help us unravel these mysteries.

## References

1. L.F. Kluskens, H.Y. Hong and L.B. Bibb ed. Radiation Biology. In: Merluce Bibbo, ed.: Comprehensive Cytopathology 25ed. Philadelphia, W.B. Saunders Co. 1997, pp 865-867.
2. Gupta AK, McKenna WG, Weber CN et al. Local recurrence in head and neck cancer: relation to radiation resistance and signal transduction. Clin Cancer Res, 2002;8: 885-892.
3. Dent P, Jarvis WD, Birrer MJ et al: the roles of signaling by the P42/p44 mitogen-activated protein (MAP) kinase pathway; a potential route to radio and chemo sensitization of tumour cells resulting in the induction of apoptosis and clonogenicity. Leukemia 1998;12:1843-1850.
4. Mendelsohn J, Baselga J: The EGR receptor family as targets for cancer therapy, Oncogene 2000;19:6550-6565.
5. Peng D, Fan Z, LuY et al: Anti epidermal growth factor receptor monoclonal antibody 225 up regulates p27 KIP1 and induces G1 arrest in prostatic cancer cells line DU 145. Cancer Res 1996;56:3666-3669.
6. Saleh MN, Raisch KP, Stachouse MA, et al: Combined modality therapy of A431 human epidermoid cancer using anti EGFR antibody C225 and radiation. Cancer Biother Rediopharm 1999;14:451-463
7. Hahn S, Bernhard EJ, Regine W, et al: A phase I trial of the farnesyl transferase inhibitor L-778, 123 and radiotherapy for locally advanced lung and head and neck cancer. Clin Cancer Res 2002;8:1065-1072.
8. J. Martin Brown. The hypoxic cell: a target for selective cancer therapy. Cancer Research, 1999;59:5863-5870.
9. Lee D.J., Spencer S., Rostok R., et al. Concurrent triapazamine and radiotherapy for advanced head and neck cancers: a phase II study. Int. J. Radiat. Oncol. Biol. Phys, 1998;42:811-815.
10. Rao GS, Murray S, Ethier SP. Radio Sensitization of human breast cancer cells by a novel Erb-B family receptor tyrosine kinase inhibitor. Int J Radiat Oncol Biol Phys 2000;48:1519-1528

## Literature Review

Compiled by Dr. P.D. Gulati

**Efficacy of tamsulosin in the medical management of juxtavesical ureteral stones. Dellabella M, Milanese G, Muzzonigro G. J. Urol 2003Dec;170(6Pt1):2202-5.**

The authors evaluated the efficacy of the alpha-adrenergic antagonist tamsulosin for conservative expulsive therapy in patients with ureteral colic due to juxtavesical stones. A total of 60 consecutive symptomatic patients with stones located in the juxtavesical tract of the ureter were randomly divided into group 1-30 who received oral floroglucine-trimetossibenzene 3 times daily and group 2-30 who received 0.4mg tamsulosin daily. The 2 groups received 30mg deflazacort daily for 10 days plus cotrimoxazole 2 times daily for 8 days and 75mg diclofenac injected intramuscularly on demand. Ultrasound followup and medical visits were performed weekly for 4 weeks. Stones passage rate and time, analgesic use, hospitalization and endoscopic intervention were evaluated. Statistical analysis was performed using the student test. The stone expulsion rate was 70% for group 1 and 100% for group 2. Mean stone size was 5.8 and 6.7mm, respectively (p=0.001). Mean expulsion time was 111.1 hours for group 1 and 65.7 hours for group 2 (p=0.020). The mean number of diclofenac injections was 2.83 for group 1 and 0.13 for group 2 (p<0.0001). Ten group 1 patients were hospitalized, of whom 9 underwent ureteroscopy, compared with none in group 2 (p<0.001, respectively). Tamsulosin used as a spasmolytic drug during renal colic due to juxtavesical calculi increased the stone expulsion rate and decreased expulsion time, the need for hospitalization and endoscopic procedures, and provided particularly good control of colic pain.

**Association of Small Dense LDL with Coronary Artery Disease and Diabetes in Urban Asian Indians: Chennai Urban Rural Epidemiology Study. V Mohan, R Deepa, K Velmurugan, K Gokulakrishnan. JAPI 2005;53:95-99.**

Earlier studies in Europeans have identified small dense LDL to be associated with coronary artery disease and diabetes. In this study we assessed the associated of small dense LDL with diabetes and CAD in Asian Indians. Study subjects were selected from the Chennai Urban Rural Epidemiology Study (CURES), a population based study on representative sample of Chennai City in Southern India. Group 1: non-diabetic subjects (n=30); Group 2: diabetic subjects without CAD (n=30); Group 3: diabetic subjects with CAD (n=30). LDL subfractions were estimated using LipoPrint LDL system. LDL subfractions 3 and above, defined as small dense LDL was summed up to determine the overall small LDL. 75th percentile of the overall small dense LDL in non-diabetic subjects was used as a cut-off for defining elevated levels of small dense LDL.

The mean age of the study subjects was not significantly different among groups. Overall small dense LDL was significantly higher in diabetic subjects with CAD (16.7 ± 11.1 mg/dl, p<0.05) and without CAD (11.1 ± 8.0 mg/dl, p<0.05) compared to non-diabetic subjects without CAD (7.2 ± 6.8 mg/dl). Small dense LDL showed a positive correlation with fasting plasma glucose (r=0.252, p=0.023), HbA1c (r=0.281, p=0.012), total cholesterol (r=0.443, p<0.001), triglycerides (r=0.685, p<0.001), LDL (r=0.342, p=0.002), total cholesterol/HDL ratio (r=0.660, p<0.001) and triglycerides/HDL ratio (r=0.728, p<0.001) and a negative correlation with HDL cholesterol (r=-0.341, p=0.002) and QUICKI values (r=-0.260, p=0.019). ROC curves constructed to predict elevated small dense LDL (9.0 mg/dl) revealed that triglycerides/HDL ratio and total cholesterol/HDL ratio had higher AUC values compared to other parameters. A triglycerides/HDL ratio of 3.0 had the optimum sensitivity (80.0%) and specificity (78.0%) for detecting elevated small dense LDL.

This data suggests that in Asian Indians, small dense LDL is associated with both diabetes and CAD and that a triglycerides/HDL ratio (3.0) could serve a surrogate marker of small dense LDL.