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Exposure to low dose ionising radiation: Molecular and clinical consequences

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ABSTRACT

This review article provides a comprehensive overview of the experimental data detailing the incidence, mechanism and significance of low dose hyper-radiosensitivity (HRS). Important discoveries gained from past and present studies are mapped and highlighted to illustrate the pathway to our current understanding of HRS and the impact of HRS on the cellular response to radiation in mammalian cells. Particular attention is paid to the balance of evidence suggesting a role for DNA repair processes in the response, evidence suggesting a role for the cell cycle checkpoint processes, and evidence investigating the clinical implications/relevance of the effect.

1. Introduction

Radiotherapy (RT) is currently utilised in the treatment of approximately half of all oncology patients during the course of their illness. The therapeutic intent of radiation oncology is to deliver a sufficiently lethal dose to the target volume to achieve local tumour control while minimising the harmful effects to normal tissues, in order to avoid treatment-related acute side effects and late morbidity. The radiation doses prescribed in current practice are based on the clinically determined radiation tolerance of the surrounding normal tissues, and a trade-off between normal tissue toxicity and tumour control is often required. A number of factors can influence normal tissue tolerance including dose, fractionation, the volume irradiated as well as individual variation in radiation sensitivity [1]. RT protocols have evolved to limit the proportion of highly radiosensitive adverse reactions to about 0.5–5% of cases [2]. Despite great clinical progress in the field there remain a small proportion of individuals who develop severe normal tissue reactions, the underlying molecular basis for which are currently imperfectly understood.

Accumulating evidence indicates that in certain tumours, RT needs to be delivered in higher than 'conventional' fractionated doses in order to achieve improved tumour control probability (TCP). To achieve this, an increasing number of RT techniques including 3-dimensional conformal radiotherapy (3D-CRT), intensity modulated radiotherapy (IMRT) and volumetric modulated arc therapy (VMAT), use multiple beams of radiation to conform the dose to the three-dimensional shape of the tumour, allowing an increased dose to be delivered to the target volume, while minimising the dose to the surrounding normal tissue. However, concern has been raised regarding the carcinogenic potential of exposing a large volume of normal tissue to such low doses of ionising radiation (IR) [3–7].

The rate of radiation-induced clonal inactivation is dose and genotype dependent [8]. Low dose hyper-radiosensitivity (HRS) is characterised by an increased sensitivity to radiation doses less than 0.3 Gy, which is followed by a more radioresistant response per unit dose between 0.3 and 0.6 Gy termed increased radioresistance (IRR). The HRS/IRR response can be defined or confirmed mathematically using the induced repair model (Fig. 1), but a modified model was recently proposed [9]. Since its identification more than two decades ago [10], it has been demonstrated *in vitro* in approximately 75% of the 50 mammalian normal and malignant cell lines tested to date (Table 1) [11]. Malignant cells lines tested included glioma [12–18], colorectal [19–24], prostate [23,25–29], bladder [22,30], cervix [23], lung [15,23,31,32], breast [15,31], melanoma [15,21,22], head and neck [33], oral [16], neuroblastoma [34], sarcoma [13,28] and Chinese hamster ovarian [35] cancer cells. The majority of normal cell lines examined were fibroblasts (Chinese hamster [20,26,36–38], rat [17], human [11,15,17,28,31,39–41], but human keratinocytes [28] and lung epithelial cells [28,42] were also tested. HRS has also been demonstrated *in vivo* in skin [43], in lung and kidney tissue,

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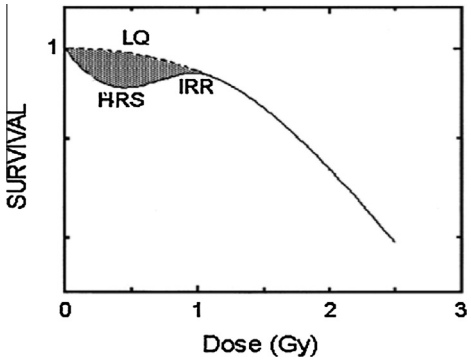

Fig. 1. Typical cell survival curve with evidence of hyper-radiosensitivity (HRS). Broken line shows low-dose extrapolation from linear quadratic (LQ) model applied to high-dose survival data. Solid line shows induced repair fit. Image adapted from [69].

metastatic tumour nodules [44], and normal human epidermis [45]. The mechanisms underlying the cell-type specific expression of HRS are still being investigated, but appear related to defective DNA repair systems and cell cycle regulation. HRS may represent an exploitable mechanism for increased tumour cell kill, and is thought to be one of the mechanisms that may increase normal tissue reactions and protect against carcinogenesis following low dose IR [46]. This review discusses the experimental data detailing the incidence, mechanism and significance of HRS in radiotherapy.

2. HRS/IRR at high doses and low dose rates

HRS appears to be a widespread phenomenon in the low dose radioresponse of mammalian cells. It has been observed in response to acute dose rate negative pi-mesons [38], high linear energy transfer (LET) radiation given at a low dose rate [47], low dose neutrons [48], protons [49] and carbon ions [41]. IRR however, is only evident after low and intermediate LET radiation exposures. Moreover, it has been demonstrated that the excess in cell killing observed at very low dose rates termed the "inverse dose rate effect" (IDRE), appears to be derived from the same radioprotective mechanism as HRS/IRR, and in fact, IDRE is thought to be a dose rate-dependent manifestation of HRS/IRR [50]. In both instances, irradiated cells experience radioprotective transitions in cell killing from hypersensitive states to radioresistant states at discrete dose rate (for IDRE) and dose (for HRS/IRR) thresholds. Leonard et al. have demonstrated that IDRE only occurs in cell lines that express HRS [50].

3. HRS and increased tumour cell kill

The clinical relevance of HRS in tumour control is a matter of debate. While HRS may increase tumour cell kill and improve the therapeutic ratio of radiotherapy, the ominous presence of tumour hypoxia in solid tumours may prevent its induction [51]. Radiotherapy delivery techniques, such as IMRT and VMAT, involve the complex arrangement of a number of external radiation beams to shape the dose distribution to the treatment volume. The therapeutic effectiveness of IMRT in the treatment of prostate cancer and head and neck tumours for instance has been well documented [52,53]. IMRT delivers dose using fields at fixed gantry angles, either dynamically or statically (step and shoot) using many beam apertures (segments) that are shaped with multileaf collimators. The number of beams used in IMRT plans can range from 5 to 15 with the daily dose maintained at 2 Gy. Similarly VMAT delivers daily doses through variable gantry rotation [54]. The standard daily dose (fraction) of radiation is thus delivered by a number of external radiation beams building up to a total dose of 2 Gy. The dose contributed by each of these beams is part of the daily fraction and represents a partial fraction (PF). The PF may be within the dose range for induction of HRS. Lin and Wu demonstrated that delivery of PF of a RT treatment such that the smaller fractions (<0.5 Gy) are delivered before larger fractions (>0.5 Gy), can induce a small increase in cell kill *in vitro* [29]. Further evidence of this phenomenon has however not been since reported and *in vivo* data is currently lacking to support potential clinical relevance.

The hypothesis that reducing the dose per fraction to doses within the HRS region may improve tumour control has been tested. *In vivo* studies utilising such ultrafractionated protocols (3 fractions of 0.4 Gy per day, interval 4 h, 7 days per week) in xenografts derived from HRS + HGL21- and T98G glioblastoma cells however failed to show improved tumour control when compared

Table 1
Prevalence of HRS by tumour origin/cell type.

	Cell origin	HRS + cell lines	HRS − cell lines	References
Malignant cells	Glioma	T98G, CAL58, A7, HGL21, U123, BMG1, U87-MG, DBTRG, MO59K, MO59J/Fus1, G5, G111, G142, G152	U373, MO59J, CL35 (subclone of G5)	[12–18]
	Colorectal	HT29, RKO	HCT116, SW48	[19–24]
	Prostate	DU145, PC3, LnCaP		[23,25–29]
	Bladder	RT112		[22,30]
	Cervix		Siha	[23]
	Lung	A549	H460	[15,23,31,32]
	Breast	MCF7		[15,31]
	Melanoma	MeWo, Be11, M4Be, A375P, SKMel2	U1	[15,21,22]
	Head and neck squamous	SCC-61, SQ20B		[33]
	Oral squamous	PECA-4451, PECA-4197		[16]
	Neuroblastoma		HX142	[34]
	Human sarcoma	HS633T (soft tissue sarcoma)	ATBr1(osteosarcoma)	[13,28]
	Chinese hamster ovarian	CHOAA8	CHO	[35]
Normal cells	Chinese hamster fibroblasts	V79, V79379A,		[20,26,36–38]
	Rat fibroblasts (oncogene-transformed)	MR4	3.7	[17]
	Human fibroblasts	MSU-1, GS3, GM0639 cells (ATM+/+, termed GMcells), MRC5, HeLax skin human fibroblast hybrid cells (CGL1, CGL3), AT22IJE-TJ EBS7YZ5 (ATM complemented)	2800T, AT5BIVA cells (ATM−/−, termed AT cells) AT22IJE-TJ EBS7 (AT)	[11,15,17,28,31,39–41]
	Human Keratinocyte		HaCAT, HPV-G	[28]
	Lung epithelial	L132		[28,42]