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Comments and Critique

Oncogenes and Radiosensitivity

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A RELATIONSHIP BETWEEN oncogenes and radioresponsiveness has been claimed, tantalisingly, by many groups. Most have investigated changes caused by high levels of expression or activation of proto-oncogenes in transfectants. There is fairly consistent evidence that the *ras* family, in certain systems, increases radioresistance [1–3] although there are reports of increased sensitivity [4] or no effect [5]. Evidence for the *myc* family is mixed, with reports of radioresistance [6] or no effect [7], although a synergistic effect with *ras* appears consistent [3, 8]. C-*raf*, derived from radioresistant human lines, confers radioresistance on transfectants [9, 10]. The association appears strong as other oncogenes, H-*ras*, K-*ras*, N-*ras*, *myc*, *fos*, *svc* or *sis*, were not found in the radioresistant transfectants. Further support for the role of c-*raf* comes from transfection of its avian analogue vmil/vmbl which also induces radioresistance [11] and, in particular, from the reversal of radioresistance by antisense c-*raf* [12]. Radioresistance has been reported with v-*abl* and c-*fms* and, at low dose rate only, v-*fos*, v-*fm*, v-*src* and v-*erb* in NIH3T3 or 32Dcl3 [6, 13]. Other oncogenes, tested to a limited degree and showing no effect, include v-*sis*, *myb*, *fos* and *fes*.

The relationship between native levels of oncogene expression on function and radioresistance of human lines is much less clear. Variant small cell lung cancer lines (SCLC-V), which are more radioresistant [14] have a greatly amplified c-*myc* gene [15]. However, specific lines have not been compared directly for the two properties. Li-Fraumeni radioresistant fibroblasts have c-*myc* levels three to eight times that of controls [10]. However, the transfection of c-*myc* from this resistant fibroblast line, 2800T into NIH3T3, does not affect its radioresponsiveness [11]. In human tumours, there is no correlation between native *ras* expression and radioresponsiveness. Transfection of *ras* into non-malignant human epithelial lines has no effect on radiation response [5, 16]. Whilst c-*raf*, transfected from radioresistant parent lines, induces resistance in transformants, levels in the parent are not amplified [9, 10]. In nine human SCLC lines examined, with a range of radiosensitivities and differing in their gene expressions, no correlation between levels of *myc*, *ras* or *raf* and radioresistance was found [17]. Warenius and co-workers in this issue examine 19 human tumour lines of various lineages, and demonstrate no correlation between the levels of protein products or *myc* or *ras* and radiation sensitivity. Whilst a correlation with c-*raf* product is seen, it is with greater sensitivity rather than resistance, in contrast to effects in transfectants [9, 10].

There are many gaps in understanding the mechanisms by which the oncogenes studied produce effects on radiosensitivity, and great problems in extrapolating from these to the role of oncogenes in radioresponsiveness of human tumours.

Transfection with any oncogene may have a variety of effects on morphology, doubling times, life span and plating efficiency. Each of these may have its own effects on radiation response. Cell cycle distribution at the time of radiation, in particular, may affect radioresponsiveness. The transfection and clonal separation techniques themselves may affect radiation response, regardless of the specific properties of the gene. Clones of increased radioresistance have been produced by neomycin resistant marker genes alone [18]. Transfection of v-k-*ras* into rat NRK cells reduces radioresistance. However, the effect is seen at both permissive and non-permissive ranges of the temperature-sensitive viral oncogene, tsk-NRK [4] which casts doubt on this being a specific effect of the oncogene product. Alternatively, many transfection experiments with a variety of oncogenes have shown no effect on radioresponsiveness, for example, v-*sis* [6], *myc* alone [3, 11] and v-*abl* [11]. Where cell cycle distribution has been investigated, there was no evidence that this would account for the changes observed [8, 19]. That oncogene effects on radiation response are specific is supported by evidence that transformed and radioresistant phenotypes are not always transferred together. Resistance persists in NIH3T3 transfectants even after reversion to non-tumorigenic forms [19]. The effects of *ras* may depend on the method of its activation in transfectants. Activation by linkage produces transformation only, whereas missense mutations produce transformation and radioresistance [2].

The precise nature of the changes in biological response after radiation is not clear, and various effects on the dose response curve have been seen. NIH3T3 *ras* transfectants showed a change in slope, that is, an effect at high doses, while *ras* and *myc* transfectants also showed effects at lower doses [2, 3, 8]. Harris' radiosensitive *ras* transfectants showed the major effect at low doses [4]. Fractionation experiments in the radioresistant R2k *ras* transfected rhabdomyosarcoma line suggested more efficient recovery [20]. Fitzgerald, in an interesting series of experiments, demonstrated increased split dose recovery in 32D-v-*abl* cells, and a specific increase in survival at dose rates of 5-cGy/min but not 116 cGy/min with transfection of v-*erb*-B, v-*abl* or v-*src* into haemopoietic stem cells [6, 13].

Changes in postirradiation cell cycle kinetics have been noted. Radioresistant REC *ras* and *myc* transfectants showed a dose

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dependent G2 arrest after radiation, greater than that seen in parent lines. The same effect was not seen in *myc* only transfectants [21]. In the same cell line, a delay in postirradiation DNA synthesis demonstrated inhibition of replicon initiation [22]. Measurement of double strand breaks demonstrated no difference in the induction or repair between parent line and transfectants [23].

The three families of *ras*-associated proteins have a variety of functions, including a regulatory role in signal transduction between cell surface receptors and the nucleus. Mutant *ras* forms have altered GTPase activity and a prolonged half life. Stauroporine, an inhibitor of protein kinases, reverses the increased delay in postirradiation DNA synthesis in REC *ras* and *myc* transfectants [21]. It is possible, therefore, that the transfected *ras* is acting by pathways not involved in normal DNA replication. The authors suggest an inhibitory factor acting on the kinase activity of $p34^{cdc2}$.

The *raf* family are also involved in signal transduction. They are serine/threonine kinases activated by phosphorylation in response to a number of stimuli, including binding of growth factors to receptors. Activation may be dependent on $p21^{ras}$ [24]. Downstream from *raf* at the nuclear level are possible effects on *myc*, which is phosphorylated on serine and threonine, regulation of *jun* and activation of c-*fos* and epidermal growth factor (EGR), genes for transcription factors that themselves control genes expressed during proliferation [25]. The ability of *raf* to confer radioresistance is related to its serine/threonine activity. Transfection of v-*mos*, which has a similar specificity also confers resistance, while v-*fes* and v-*abl*, which phosphorylate tyrosine, do not [11]. Ruggiero and colleagues [26] demonstrated a common pathway for several oncogenes implicated in radioresistance, acting through protein kinase C, activated by a rise in diacylglycerol.

A further development on the role of *ras* and *raf* in the same transduction pathway is the demonstration of aberrant catenation of topoisomerase II in NIH3T3 c-*raf* transfectants and Li-Fraumeni fibroblast lines. A correlation was demonstrated between increased topoisomerase II activity and radioresistance [27]. Tyryosine kinases, such as v-*fes* and v-*abl*, which do not alter radioresistance [11] have no effect on topoisomerase II activity.

The effect of radiation on gene expression has been reviewed by Weichselbaum and colleagues [28]. Early response genes demonstrated include nuclear *jun* and *fos* (activator protein-1 genes), EGR and *rel* transcription factors. The activation pathways can be activated by a wide variety of stimuli, and they in turn activate a large number of secondary response genes. It is not yet clear how abnormal activation of the *ras-raf* transduction pathways might alter these normal responses, but there may be a link through *jun*, *fos* and EGR.

Warenius and colleagues [29], by examining a larger bank of human cells than ever reported previously, and by correcting for cell cycle effects, have added to the debate on the role of oncogenes in determining resistance in tumour lines. That *ras* and *myc* have no demonstrable association is consistent with what previous human data is available. That c-*raf* may have a role is also consistent with what is known, but surprisingly, the association is the reverse of that reported previously.

As the authors point out, measurement of c-*raf* product gives no information on its function, and it is possibly with function rather than level that further progress is to be made.

Other resistant parental lines do not show amplification [9, 11], and whilst there is amplified expression in transfectants this is of abnormal transcripts. The transfected gene has been truncated. Neither abnormalities of gene or transcript structure are found in the parent [9]. Where c-*myc* has been implicated,

Table 1. *Oncogene transfection and radioresponsiveness*

Oncogene	Cell	Effect alone	Comment	Author	Reference
ras	NIH3T3	R		Fitzgerald 1985	1
ras	NIH3T3	R		Sklar 1988	2
ras	NIH3T3	S		Harris 1990	4
ras	NIH3T3	R	LDR	Fitzgerald 1990	6
ras	REC	R	syn *myc*	Ling 1989	8
ras	REC	R	syn *myc*	McKenna 1990	3
ras	HBL100	No effect		Alapelite 1990	5
*ras**	NIH3T3	R		Samid 1991	19
ras	R2k	R		Hermens 1992	20
ras	MulLu	No effect		Russell 1992	7
myc	REC	R	syn *ras*	Ling 1989	8
myc	REC	No effect	syn *ras*	McKenna 1990	3
myc	NIH3T3	R	LDR	Fitzgerald 1990	6
myc	32Dcl3	R		Fitzgerald 1990	6
myc	MvlLu	No effect		Russell 1992	7
raf	SQ20B	R	Reversed by AS	Kashid 1989	12
raf	NIH3T3	R		Chang 1987	10
v-*abl*	NIH3T3	R	LDR	Fitzgerald 1990	6
v-*abl*	32Dcl3	R	LDR	Fitzgerald 1990	6
c-*fms*	32Dcl3	R		Fitzgerald 1990	6
v-*fms*	NIH3T3	R	LDR	Fitzgerald 1990	6
v-*mos*	NIH3T3	R		Pirollo 1989	11

REC, rat embryo cells; HBL100, human mammary line; R2k, rat rhabdomyosarcoma; 32Dcl3, haemopoietic progenitor line; MvlLu, mink lung epithelial line; SQ20B, laryngeal cancer line; NRK, rat line; R, resistant; S, sensitive compared to parent line; LDR, at low dose; *, over-expression of proto-oncogene; syn, synergistic with; AS, antisense.

amplification of germ line *myc* has been demonstrated, although additional hybridising bands are seen in some lines [15]. Although amplification of *ras* has been demonstrated in transfectants [4], the more usual abnormality is a missense mutation. Radioresistance is not related to number of copies [2].

Radiation response, therefore, appears to be dependent on mutant rather than amplified gene. Correlations between levels and radioresistance are now unlikely to cast further light. Warenius and colleagues (pp. 369–375) wish to examine the effect of transfected constructs of the c-*raf* gene. In addition, some measure of function of gene products downstream of *raf*, and of the effect of antisense *raf* might add further to this fascinating story.

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