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Editorial Comment

Editorial comment on 'A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy' by Schmitt *et al.*

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'What are the mechanisms of intrinsic drug resistance in solid tumours, and how might they be circumvented?' These are key questions for molecular oncologists, for clinicians and patients.

For many years, it was assumed that the interaction of a DNA damaging drug with its target would yield a lethal lesion, and that determinants of intrinsic drug resistance should therefore be sought upstream of this interaction, in drug metabolism or drug transport mechanisms. It is now apparent that cellular responses *in vitro* to a given DNA lesion can include necrosis, mitotic catastrophe, apoptosis, prolonged cell cycle arrest or even unrestrained growth. The response depends on the cellular genotype and, specifically, on the integrity of response pathways *downstream* of the drug–target interaction. Despite this progress in the laboratory, the proof that downstream events determine therapeutic responses in the clinic remains elusive.

A seminal series of papers from Scott Lowe's laboratory represent a major step in this direction. Lowe and colleagues prove conclusively that downstream determinants of apoptosis profoundly influence the response to therapy *in vivo*. They also demonstrate, for the first time, that prolonged drug induced growth arrest can result in prolonged disease stability whilst providing a residual pool of viable malignant cells from which late relapsing clones may ultimately emerge. These papers have mapped out new challenges and new opportunities for the oncologist of the future.

Utilising the Eμ-*myc* transgenic murine model of B-cell lymphoma (in which myc overexpression drives lymphomagenesis), Lowe and colleagues have developed a rapid technique for the introduction of defined compound genetic lesions, allowing study of resulting phenotypes in a well-controlled fashion *in vivo* [1]. Primary lymphoma or haematopoetic stem cells, isolated from mice of known genetic background (Eμ-*myc* crossed with *P53*-null, or *ARF*-null or *ARF*/*INK4a*-null), are retrovirally transduced *ex vivo* with a gene of interest and reintroduced into the tail veins of syngeneic recipients. The transduction process itself does not alter the subsequent behaviour of the resulting lymphoma, so that controls transduced with the green fluorescent protein (GFP) gene alone yield lymphomas histopathologically indistinguishable from the parent lymphoma. Introduction of a gene of interest along with GFP in a bicistronic retroviral vector allows study of the effect of that gene on tumour behaviour by the elegant non-invasive means of whole-animal fluorescence imaging.

An early paper in the series demonstrates conclusively that overexpression of the anti-apoptotic protein bcl-2 produces a multi-drug resistance phenotype in primary lymphomas *in vivo* [2]. This effect is suppressed by prior serial passage of the lymphoma lines *in vitro*, because the cells acquire multi-drug resistance merely as a consequence of prolonged cell culture. Furthermore, the effect of bcl-2 is completely obscured in standard clonogenic assays of drug sensitivity, because bcl-2 suppresses drug-induced apoptosis, but not prolonged growth arrest, both equally efficient means of reducing clonogenicity.

These observations shed some light on why assays of drug response in established cell lines or xenografts derived from solid tumours have failed in the past to consistently predict clinical response, and indeed have often provided contradictory results. In general, such models simply cannot recapitulate conditions pertaining *in vivo*.

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Building on their early results, the authors demonstrate that bcl-2 overexpression can completely suppress the selective pressure for loss of *TP53* during lymphomagenesis [3]. On a *TP53* ± heterozygote background, Eμ-*myc* driven lymphomas invariably acquire loss of the remaining wild-type *TP53* allelle, leading to a *TP53*-null rapidly progressive malignancy. Bcl-2 overexpression in the TP53 ± background leads to the emergence of a similarly aggressive malignancy, but without loss of the wild-type *TP53* allele. The *TP53*-null lymphomas rapidly develop aneuploidy and chromosomal instability, whereas the bcl-2 overexpressing *TP53*± lymphomas retain genetic stability. This remarkable finding indicates that, in this setting at least, genetic instability is merely a bystander effect of *TP53* loss. It is the pro-apoptotic functions of *TP53* which drive the selective pressure for *TP53* loss during lymphomagenesis.

In most other respects, the *TP53* null lymphomas are phenotypically similar to the bcl-2 overexpressing *TP53*±lymphomas, until one comes to examine response to chemotherapy [4]. Thereupon a dramatic difference in behaviour is revealed. Lymphomas expressing wild-type p53 protein respond well to therapy and are often cured. The *TP53*-null variants rapidly relapse, and the mice die quickly. Overexpression of bcl-2 in *TP53* null tumours makes no difference to response or survival. However, overexpression of bcl-2 on the *TP53*± background has a profound effect. The immediate response is no longer observed, nor do the tumours rapidly progress. The disease enters a stable state, lasting many weeks, but ultimately terminating in a lethal late relapse. During the stable phase, from as early as a few days after therapy, bcl-2 overexpressing *TP53*±cells are arrested (no bromo deoxyuridine (BrdU) incorporation or Ki67 staining) and express markers of cellular senescence promyelocytic leukaemia protein (PML), SA-β-gal). Upon relapse, the emergent malignant clones are often found to have lost the remaining wild-type *TP53* allele.

In a 'tour de force' of transgenic techniques, Lowe and colleagues have proceeded to identify the cooperative interaction of wild-type p53 with p16 INK4a as a critical genetic determinant of this drug-induced senescence. In the process, they illustrate that the genotype of clones which relapse after therapy depends in large part on the genetic background of the tumour from which they arise. Thus the mechanisms of intrinsic drug resistance, and the acquisition of new resistance mechanisms upon relapse, are profoundly influenced by the tumorigenic pathway giving rise to the primary disease. Furthermore, these influences can be merely the result of bystander effects, exemplified beautifully at the *ARF*/*INK4a* locus. At this locus, exons 2 and 3 are shared by the product of the *ARF* gene and by the product of the *INK4a* gene (although in different reading frames). On an *ARF* heterozygote background, Eμ-*myc* driven lymphomas invariably acquire loss of the second *ARF* allele (which incidentally also relieves selective pressure for *TP53* loss, by virtue of the role *ARF* plays in myc driven induction of p53-mediated apoptosis). When loss of the second *ARF* allele occurs by mutation of exon 2, an ARF-null, but *INK4a* heterozygote lymphoma arises. The *INK4a* heterozygote state is silent and in itself confers no selective advantage during lymphomagenesis. However, in this *INK4a* heterozygote state (induced as a bystander event by selective pressure for *ARF* loss), relapse after treatment is rapid and is accompanied by loss of the remaining wild-type *p16 INK4a* allele. In contrast, when the second allele of ARF is lost in a manner which spares *INK4a*, response to treatment is excellent and durable. Further elegant experiments directly demonstrate a lack of selective advantage for *INK4a* loss during lymphomagenesis, but a complete dependence of the drug-induced senescence response on p16 INK4a expression in a *TP53* wild-type background. In conclusion, disruption of growth arrest (e.g. by *INK4a* loss), and disruption of apoptosis (e.g. by bcl-2 overexpression) act independently to promote drug resistance, downstream of the DNA lesion.

So what of opportunities and challenges? On an optimistic note, the authors have demonstrated two separate bystander effects (genetic instability in *TP53*-null cells and *INK4a* loss) neither of which is selected for during lymphomagenesis, but each of which nevertheless influences therapy (in these cases producing resistance). Precisely because such bystander effects are unselected, it might be hoped that under different circumstances, at a different locus, or under different therapy, similar bystander effects might instead give rise to *enhanced* sensitivity and an exploitable therapeutic opportunity. In any event, it is clear that a detailed understanding of the genetic status of a given tumour will provide an opportunity to individualise and thus optimise primary therapy. It may even be possible to predict likely genetic mechanisms of relapse (and thus select appropriate combination or sequential therapies). For example, targeting bcl-2 overexpression in tumours provides an opportunity for rational drug design. Clinical trials using bcl-2 antisense are currently underway [5] and, in combination with chemotherapy, might provide a means for switching tumour responses from prolonged arrest (with ultimate relapse) to apoptosis (with cure).

The challenges include cataloguing genetic determinants of known and future therapeutic agents, and developing clinically feasible means of characterising the genetic status of a given tumour. Complexities abound. As an example, are all *TP53* mutations functionally equivalent to the *TP53*-null state? How much genetic detail is required? Do genomic lesions need to be characterised in detail (as implied by the *ARF*/*INK4a* story above)? Or will expression profiles be adequate in practice? Encouragement has recently come from that most well understood of malignancies, childhood acute

lymphoblastic leukaemia (ALL) [6]. It seems that a finite number of different forms of ALL can be defined at the level of gene expression, utilising rapid throughput microarray technology applicable to clinical specimens. However, as always, the devil is in the detail. Even in their simplified murine lymphoma model, with well defined genetic background, Lowe and colleagues frequently encountered genetic heterogeneity in the resulting lymphomas (and deployed considerable experimental ingenuity to circumvent this confounding factor). It seems probable that advanced malignant solid tumours in humans will harbour multiple genetically different subclones. The task of the clinician charged with matching therapy to each of these clones in a single patient will be formidable.

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