

Tumour Cell Death

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The aim of our group is to understand the factors regulating programmed cell death in cancer cells. Since it is known that inhibition of cell death mechanisms is a common event in tumour development, this poses problems for many forms of chemotherapy that utilise cell death pathways, leading to drug resistance. We are investigating both known cell death regulators as well as searching for novel proteins that control cell death and chemosensitivity. We envisage that the knowledge gained from our studies will be translated and lead to the improvement of existing clinical regimens or new targets for therapeutic intervention.

Oncogene-induced sensitisation to death requires induction of E2F1

Understanding differences in drug sensitivity between normal and cancerous cells is fundamentally at the core of many forms of chemotherapy. Many of the insights into this issue have come from the study of transforming proteins from DNA tumour viruses. In particular, the E1a protein from transforming strains of adenovirus has led to the identification of many of the proteins that are now known to be intrinsically involved in tumour development. E1a is also well characterised as a factor that sensitises cells to chemotherapy-induced death. Principally, this involves E1a's capacity to bind members of the retinoblastoma (RB) family. As a result, the cellular transcription factor E2F1, which the pRb member of the RB family normally keeps in check, becomes deregulated. In this de-repressed state, E2F1 can either stimulate cell death directly through the activation of apoptotic target genes or can signal cell death via the tumour suppressor p53.

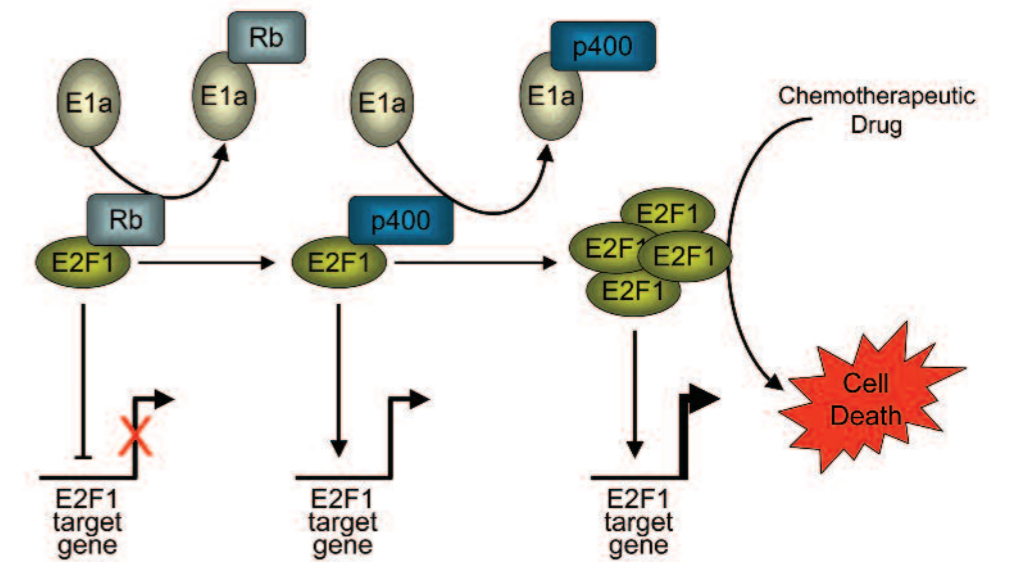
Our studies of E1a-mediated transformation revealed that E1a not only deregulates E2F1 but also causes levels of the E2F1 protein to increase. This was not reflected in an increase in E2F1 mRNA indicating that E1a affects E2F1 protein stability. Interestingly, different to the situation with E2F1 deregulation, the effects of E1a on E2F1 protein levels were found to be

independent of pRb. Analysis of a panel of deletion mutants of E1a revealed instead that binding of E1a to the cellular p400 protein was involved. Mutants of E1a that cannot bind p400 were unable to increase E2F1 protein levels and were also found to be impaired in their ability to sensitise cells to chemotherapy-induced death. The fact that this effect was specifically due to a lack of interaction with p400 was confirmed when we observed that E1a mutants lacking p400 binding could be rescued in their ability to sensitise cells to death by knockdown of p400 by RNA interference. The knockdown of p400 caused an increase in E2F1 protein levels in the absence or presence of E1a, indicating that other functions of E1a are not required for this effect. Sensitisation to death, however, could not simply be achieved by knockdown of p400 indicating potentially that deregulation as well as upregulation of E2F1 are required for this effect. These studies therefore revise the long-standing dogma of how viral oncoproteins sensitise cells to death via E2F1 and potentially highlight new avenues for development of strategies to cause tumour cell death.

The stability and apoptotic activity of Puma is regulated by phosphorylation

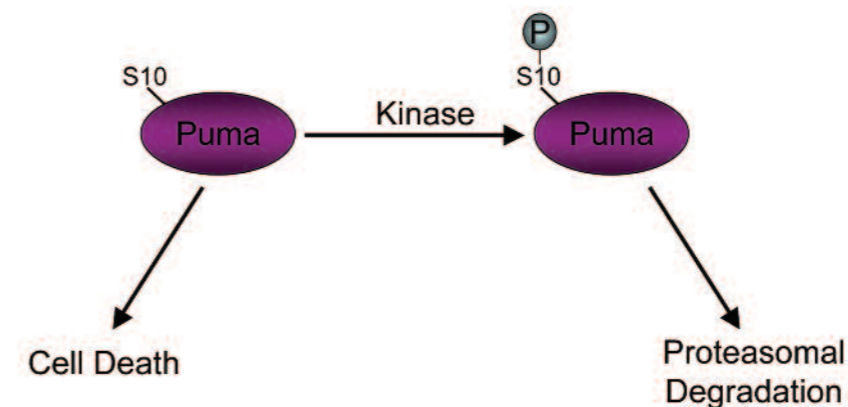
Regulation of programmed cell death often involves permeabilisation of the outer mitochondrial membrane, which members of the Bcl-2 family regulate. Puma is a potent

Figure 1
The viral oncoprotein E1a binds to members of the retinoblastoma family causing deregulation of E2F1 and sensitivity to chemotherapy-induced death. We have recently shown that E1a also increases the levels of E2F1 via p400 (potentially through removal of p400 from E2F1) and this is also required for full drug sensitivity.



pro-apoptotic member of the Bcl-2 family and is activated by numerous factors including p53, p73, E2F1 and FOXO3a. In living cells Puma activity must be tightly controlled and since Puma levels can be induced without cell death induction, we postulated that Puma might be subject to negative regulation by post-translational modification. To analyse this, we first tested if Puma was a phosphoprotein such that its activity may be subject to kinase control. This revealed that in growing cells, Puma is phosphorylated on multiple sites with the principal site of phosphorylation being serine 10. Mutation of serine 10 to alanine did not affect the ability of Puma to bind to anti-apoptotic members of the Bcl-2 family nor did it affect the cellular localisation of Puma. Mutation of serine 10 to alanine did, however, affect the rate of proteasomal degradation of Puma in a manner independent of caspases and macroautophagy. The increase in Puma stability and steady-state protein levels in the absence of serine 10 phosphorylation led to an enhanced ability of the protein to induce programmed cell death. Although the signals that lead to changes in Puma phosphorylation at serine 10 are yet to be

Figure 2
The BH3-only protein Puma is a potent inducer of cell death downstream of multiple signalling pathways. The activity of Puma must therefore be tightly controlled. We have found that the proteasomal degradation of Puma and as a result its apoptotic potential are controlled by phosphorylation at serine 10.



determined, due to the highly pro-apoptotic power of Puma we believe these findings may constitute a novel critical control point in cell fate decisions.

p53 and NF-κB coordinately regulate expression of Noxa and p53AIP1

Two of the key factors regulating programmed cell death within the cell are the p53 and NF-κB transcription factors. Previous work from ourselves and others has reported interplay between p53 and NF-κB in the control of programmed cell death. In our own studies, we found that activation of p53 leads to an increase in the DNA binding of the canonical NF-κB heterodimer containing the NF-κB family members p65 and p50. Retention of NF-κB in the cytoplasm with a constitutively active form of the endogenous NF-κB inhibitor, IκBα, surprisingly also led to inhibition of p53's capacity to induce programmed cell death. In an attempt to understand the mechanism underlying this effect, we decided to profile the activation of p53 target genes either in the absence or presence of the dominant-negative form of IκBα. These studies revealed that activation of the majority of p53 targets, including well-characterised targets such as p21 and hdm2, were completely unaffected by inhibition of NF-κB. In contrast, the ability of p53 to activate the mRNA levels of the pro-apoptotic target gene, p53AIP1 was markedly reduced when NF-κB was inhibited. Interestingly too, the levels of Noxa protein (a pro-apoptotic member of the Bcl-2 family), but not Noxa mRNA were also reduced upon inhibition of NF-κB. These data therefore highlight two new control points at which NF-κB can contribute to p53-mediated programmed cell death.

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