

Tumour Suppression

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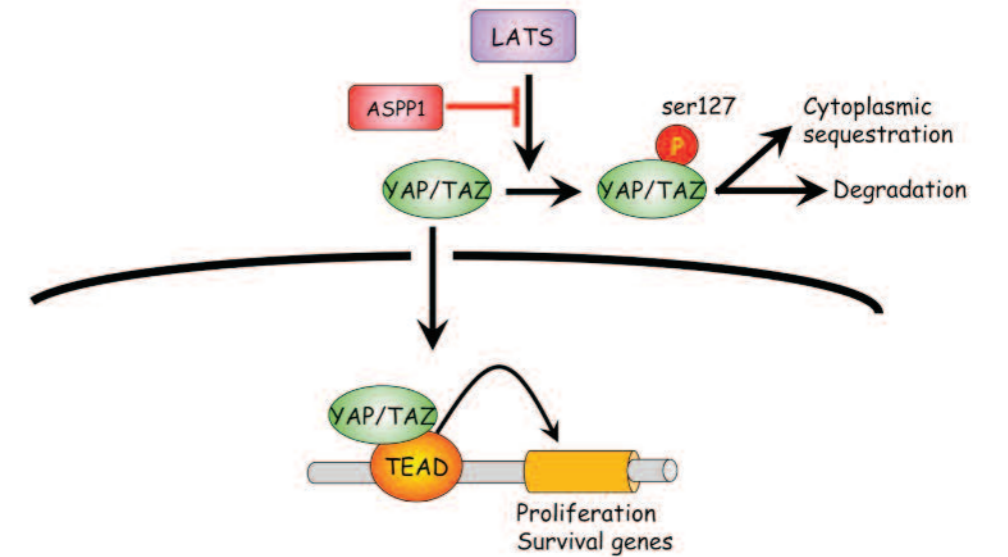
Our main focus is to study the regulation and functions of p53, an important tumour suppressor protein that prevents the outgrowth and survival of cells undergoing malignant transformation. During the course of these studies, we and others have found that p53 also plays a role in allowing cells to survive and adapt to lower levels of constitutive stress that occur during normal growth and development. p53 therefore plays a dual role in protecting cells from becoming malignant – helping to repair or prevent moderate or transient damage, while eliminating irreparable or permanently damaged cells. These activities of p53 reflect, to a large part, the ability of p53 to function as a transcription factor and regulate the expression of genes in response to various stress stimuli. We are interested in determining the function of these p53-inducible genes, and to understand how they help to mediate the growingly complex responses to p53 activation. Of particular interest during the past year have been the mechanisms that can regulate the response to p53, and how p53 target genes are selected under different conditions.

Regulation of apoptosis by ASPP1

The ASPP family of proteins was identified through interactions with p53, and shown to modulate p53's transcriptional activity. ASPP1 and ASPP2 are very similar, and both have been shown to help promote the ability of p53 to drive the expression of apoptotic target genes. The mechanism through which these ASPP proteins function appears to be by dissociation of the third member of the family, iASPP that normally inhibits expression of the apoptotic genes regulated by p53. These important actions of the ASPP family reflect the activity of these proteins in the nucleus of the cell, where p53-driven transcription takes place. We were therefore intrigued by the observation that ASPP1 was predominantly localised to the cytoplasm in many different cell lines, suggesting that there may be additional functions for this protein. By looking at the regulation of gene expression in cells depleted of ASPP1 we found that in addition to the modulation of p53-

dependent transcription, ASPP1 could also influence the activity of YAP/TAZ – a member of another family of transcription factors that has been shown to regulate cell proliferation and apoptosis. Interestingly, YAP/TAZ can shuttle between the cytoplasm and nucleus and so represented an attractive candidate for the mediation of a cytoplasmic activity of ASPP1. We were able to show that ASPP1 can inhibit the interaction of YAP with LATS1, a kinase that phosphorylates YAP and promotes cytoplasmic sequestration and protein degradation. This function of ASPP1 therefore enhances nuclear accumulation of YAP and YAP-dependent transcriptional regulation (Fig. 1). The consequence of YAP activation by ASPP1 is to inhibit apoptosis, in part through the downregulation of Bim expression, leading to resistance to anoikis and enhanced cell migration. These results reveal a potential oncogenic role for cytoplasmic ASPP1, in contrast to the tumour suppressive activity previously described for

Figure 1
Regulation of YAP/TAZ activity by cytoplasmic ASPP1, which prevents the phosphorylation of YAP/TAZ by LATS thereby promoting stability and nuclear accumulation of YAP/TAZ and the expression of survival genes.



nuclear ASPP1. Such a dual role for ASPP1 suggests that it will be important to understand what regulates the subcellular localisation of the protein, and a study from the group of Moshe Oren, published in concert with our paper, showed that phosphorylation of ASPP1 by LATS2 could promote nuclear accumulation and activation of apoptotic target gene expression by p53. Intriguingly, this effect was seen in response to Ras activation - a common oncogenic alteration in cancer - and could be inhibited by high expression of YAP. Taken together, the two papers reveal a complex and intimate interdependent regulation between ASPP1, YAP/TAZ and LATS. It seems that the outcome to ASPP1 function can switch between p53-mediated tumour suppression (when ASPP1 is driven to the nucleus by LATS phosphorylation) and p53-independent oncogenic activity (when cytoplasmic ASPP1 controls the ability of LATS to phosphorylate and activate nuclear accumulation of YAP/TAZ). Predicting the effect of ASPP1 inhibition on tumour development is therefore complicated, and further studies will be required to address this interesting question.

Functions of mutant p53

Many animal studies have shown that loss of p53 can result in a high susceptibility to cancer. p53 is frequently mutated in human cancers but interestingly these mutations lead to the expression of a mutated p53 protein, generally carrying a single amino acid substitution in the central DNA binding domain. These mutant p53 proteins not only lose wild type tumour suppressor functions but also acquire an ability to promote invasion and metastasis. Last year, we initiated studies aimed at investigating the mechanisms by which mutant p53 can function and found that it is able to promote the activation of integrin and EGFR trafficking that depends on Rab-coupling protein (RCP) and which results in constitutive activation of EGFR/integrin signalling. This activity appeared to correlate with the ability of mutant p53 to inhibit

the activity of p63, a p53 family member. In further studies, we have extended these observations by showing that mutant p53 can also promote the ability of cells to invade towards hepatocyte growth factor (HGF), although invasion towards other growth factors, such as IGF-1 and PDGF was not influenced by mutant p53 expression. HGF is the ligand for the tyrosine kinase receptor MET, a protein that has a clear influence in driving invasion and metastasis of cancer cells. In addition to showing increased invasion, expression of mutant p53 also induced cell scattering, a response to MET signalling that results in loss of the normal contacts between cells, and is thought to correlate with the ability of cells to move away from a primary tumour mass and begin the process of metastasis. As with EGFR, the ability of mutant p53 to drive signalling through MET was dependent on integrin and RCP, and could be recapitulated by knockdown of p63 expression. Taken together the results suggest that mutant p53 functions by inhibiting p63, which engages an RCP-dependent activation of oncogenic growth factor receptors such as EGFR and MET. Studies in mouse models have suggested that these activities of mutant p53 may not play a role in the generation of a primary tumour, which seems to depend on loss of wild type p53 function. However, the acquisition of a metastatic phenotype is greatly enhanced by expression of mutant p53, and is likely to reflect - at least in part - the ability of mutant p53 to promote the activation of the signalling cascades downstream of growth factor receptors. Small molecule inhibitors of several receptor tyrosine kinases are in clinical use, and our results suggest that the expression of mutant p53 may indicate effectiveness of this line of therapy, even in cancers without obvious amplification or alteration of the receptors themselves.

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