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RESEARCH HIGHLIGHT

p53 and NFκB: fresh breath in the cross talk

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Adenocarcinomas are a major subtype of non-small cell lung cancers (NSCLC) that account for up to 80% of all lung cancers world-wide. It is known that up to 30% of adenocarcinoma patients have oncogenic (tumor-driving) mutations in K-ras and up to 50% have loss of function in the tumor suppressor p53 [1]. Both these alterations, namely activation of Ras [2, 3] and loss of wild type p53 function, have been reported to activate the transcription factor nuclear factor-κB (NFκB) in independent set of studies across many laboratories [4]. The NFκB pathway is known to be key for various cellular and molecular events especially in innate and adaptive immunity. IκB proteins inhibit NFκB function by preventing NFκB DNA binding. The rate-limiting step in the activation of NFκB is the phosphorylation of the IκBs, which is mediated by the IκB kinases (IKK1 and IKK2). Recently it has become evident that increased activity of the IKKs and NFκB are frequently seen in many types of cancers in humans. However, is NFκB hyperactivity the driver or the passenger in many of these human cancers still needs to be investigated.

Unlike the evidence for NFκB function in Ras-mediated oncogenesis, which is scanty, the interplay between NFκB and p53 pathways has been well documented in response to a large number of physiological contexts such as DNA damage [4]. A general consensus is that p53 and NFκB negatively regulate each other's activity. This is achieved by competition for limited co-factors such as p300/CBP or in the case of p53, it has been documented that NFκB also negatively regulates its stability [4]. Mice with a conditional deletion of IKK2 in the intestine show elevated p53 level and so do fibroblasts null for IKK1/2 and for the p65 subunit of NFκB. On the other hand, p53-null fibroblasts show no increase in levels of NFκB family of proteins nor do they show overt activation of NFκB target genes. These results show that p53-mediated repression of NFκB is highly context-dependent [5, 6] and operates at levels of protein-protein interaction and protein modification rather than protein stability.

Although the role of NFκB in tumorigenesis has been investigated previously using mouse models, no definitive role of a functional crosstalk between p53 and NFκB in the initiation or progression of tumors has emerged. The results of Myelan *et al.* are the first to document that NFκB is activated, in human or mouse lung cancer cells with both loss of wild type p53 and gain of Ras [7]. More importantly, NFκB activity is required for lung cancer cells with loss of wild type p53 and gain of Ras to survive and grow. Since persistent nature of the NFκB activity is required for the tumors to grow, it appears that the tumors described in the study are addicted to continuous NFκB activity. This conclusion was reached as inhibition of NFκB by a dominant negative IκB protein led to significant reduction in tumor size and growth rate over time. This situation is parallel to the situation in cancers which are addicted to activities of factors such as myc and Raf [8-10], and loss or diminishing the function of these factors causes the tumor cells to grow at a diminished pace. Myelan *et al.* use nuclear accumulation of p65 as a measure of NFκB activation in this study. However, it is known that merely driving NFκB (p65 subunit) to accumulate in the nucleus is not enough for it to function as a transcription factor. NFκB needs to be modified covalently to function as a transcription factor [11]. The status of NFκB modifications in the lung cancer cells has not been investigated by Myelan *et al*.. Taken together with the fact that differentially modified NFκB could actually repress (rather than activate) transcription and that classical NFκB genes are found not to be upregulated by Myelan *et al*., it is difficult to surmise whether the increased p65 accumulated in the nuclei of the cancer cells under study works as a transcriptional activator or repressor of certain key target genes required for tumor initiation and/or progression. On the other hand, an interesting hitherto undocumented cross talk between p53 and the c-Rel component of NFκB was also noted in these tumor cells.

So what does nuclear accumulation of NFκB in conditions of p53 loss and gain of Ras achieve? NFκB is known to regulate over 200 protein

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genes and even microRNAs as a part of its physiology. These NFκB target genes include genes for cytokines and growth factors, for cell surface and cell adhesion molecules, for cell growth and division and most important genes required for protection against apoptosis. But a surprise in the study was that the genes commonly regarded as mediators of NFκB-mediated protection against apoptosis such as Bcl2, Bclx and Xiap were not found to change dramatically in the lung cancer cells again highlighting that the NFκB-dependent effects like those of p53 are also very context-dependent. Instead, the list of genes that did change makes one believe that it is more likely that NFκB-mediated effects in the type of lung cancer under investigation might be due to its effects on inflammation or metabolism. Interestingly, p53-mediated repression of NFκB had a clear effect on Glut3 and pursuing this line of research will indeed uncover exciting new mechanisms by which NFκB and p53 operate in the context of human cancers.

It is well regarded that the tumor suppressor p53, either through its role as a transcription factor or as a molecule that regulates mitochondrial function and microRNA synthesis promotes either cell death or cell cycle arrest under most physiological settings. The role of NFκB in conjunction with p53 under various settings has been a bit ambiguous and context-dependent [4]. The differences in the functioning of NFκB could be attributed to its differential interaction with p53 and the fact that under some (as reported by *in vitro* studies) conditions, NFκB and p53 can also function co-operatively. Given that both NFκB and p53 function as transcription factors, it is possible that genes most likely to change during loss of p53 and activation of NFκB are the ones that are either directly or indirectly co-regulated by these two factors. Apart from the canonical NFκB pathway, which is most frequently found mutated in cancers and was evaluated by Myelan *et al.,* evidence that the non-canonical NFκB pathway can also regulate p53 function through an alternative mechanism has also emerged. It was shown that p52 subunit of NFκB could be recruited by p53, to its target promoters, where, depending upon the gene, it could either repress or stimulate p53 transcriptional activity [4]. These results open the possibility that a network of both canonical and non-canonical NFκB pathways can functionally intersect with p53 function. Hence it can be hypothesized that aberrant activation of both these pathways in tumors could impinge on tumorigenesis through modulation of p53 activity and hence activity of both these pathways should be evaluated in future studies. Significantly, a recent large scale genomic sequencing study found multiple mutations in IKKα (which regulates non-canonical NFκB pathway) across many human cancers [12], and constitutive activation of IKKα and phosphorylation of CBP in lung cancers which were reported to play a role in the p53 and NFκB cross talk [4]. Myelan *et al.,* did not describe the mechanism of how p53 loss results in NFκB activation. On the contrary, p53-mediated NFκB activation has also been reported through activation of rsk [13]. In the future, it will be important to find the circumstances under which NFκB activation and subsequent p53 suppression is dominant over p53 activation and subsequent NFκB suppression.

Given the parallels between the regulation of NFκB by p53 status in human and mouse lung cancer cells, it is probably important to justify that activators of p53 and inhibitors of NFκB could be used as adjuvant in treatment of this form of cancer. Several natural products and approved or candidate drugs are known to activate p53 and hence presumably activate its tumor suppressor function. Similarly, many novel or clinically approved drugs and natural compounds are now proven to also inhibit NFκB raising the exciting possibility that NFκB inhibition is at least in part responsible for their efficacy. While combining drugs that have the desirable property of either inhibiting NFκB or activating p53 individually is ideal, it will be the goal of the pharmaceutical companies to find single entities that simultaneously activate p53 and repress NFκB. Indeed, there already exist a number of drugs which simultaneously activate p53 and also inhibit NFκB [14, 15]. These drugs present a wonderful opportunity to treat cancers like the type described by Myelan *et al.,* or other cancers which show a deregulation of both these pathways [16]. Better understanding the pharmacology of these drugs and modifying them suitably can be a robust way of hitting the two targets at the same time and hence more effectively treating some type of cancers especially that of lung.

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