

Regulation of gene expression by oncoproteins

Cancer development results from the accumulation of mutations which lead to uncontrolled and unscheduled proliferations. One mutation in a key protein can be enough to initiate tumourigenesis; the two most widely studied of these proteins are p53 and pRb (Retinoblastoma). As of March 2010, 25,000 mutations were known to affect the function of p53, and there were similar number of publications documenting them. p53 is mutated in up to 50% of human malignancies; thus a large amount of research funding has been channelled into studying the area (Petitjean et al., 2007).

The basic function of p53 is as a receptor of genotoxic stresses (Zuckerman et al., 2009). DNA damage, nucleotide depletion, heatshock, hypoxia, or pH change can all lead a large increase in p53 expression, and a wide range of downstream effects. The most well characterised downstream effects are apoptosis, DNA repair and cellular senescence. Intensity, duration and origin of the stress signal play a part in choosing which pathway is activated. Qualitative status of p53 may contribute to preferentially activating a particular pathway; the conformation, localisation, activity and stability status of p53 can be altered by a number of factors, perhaps leading to one pathway in favour of another (Zuckerman et al., 2009).

The discovery of the ASPP family of p53 regulators by the Xin Lu research group was the first step in understanding how p53 pathway selection may occur (Samuels-Lev et al., 2001, Trigiante and Lu, 2006). The ASPP family is named after the characteristic Ankerin repeat domains, SH3 domains and proline rich domains found at the C-termini of all three family members (Trigiante and Lu, 2006). ASPP1 and ASPP2 also have conserved N-termini, and both function as stimulators of apoptosis. The third family member, iASPP, is a negative regulator of p53 induced apoptosis (Sullivan and Lu, 2007). iASPP is the most evolutionarily conserved member of the family; a recent study which expressed the human iASPP protein in *C.elegans* reported that function was identical (Bergamaschi et al., 2003). The most unique characteristic of the ASPP family is how they interact with p53. Most

partners bind to the trans-activation domain at the N-terminus; however ASPP interacts with the DNA-binding domain of p53 (Trigiante and Lu, 2006). This suggests that ASPP may be able to directly influence the affinity to which p53 binds promoters of downstream genes, thus influencing which pathway p53 can mediate. The DNA binding domain of p53 is also the most frequently mutated (Petitjean et al., 2007).

The discovery of ASPPs and other regulators of p53 have changed the focus of cancer research; instead of studying p53 directly, oncoproteins which interact with it have become the centre of attention.

Recent observations have shown ASPPs to be down regulated in hepatocellular carcinomas (HCCs), a cancer with only a 30% mutation rate of p53 (Petitjean et al., 2007). Independent findings have also shown an increase in recruitment of DNA methyltransferases (DNMTs) to tumour sites of HCCs positive for the Hepatitis B virus (HBV) (Lee et al., 2005). Together, these observations indicate that an increase in methylation mediated by HBV could be responsible for the downregulation of ASPPs (Zheng et al., 2009). Zhao et al tested this hypothesis with a combination of cell culture and primary tissue samples (Zhao et al., 2010). Initial characterisation showed that in a range of 8 cell lines isolated from HCCs, all had a decrease in ASPP expression in comparison to a normal hepatic liver cell line, and this down regulation could be reversed by treating the cell lines with 5-aza-2'-deoxycytidine, a demethylating agent. In 51 paired samples taken from HBV positive HCC patients, methylation of ASPP promoters and downregulation could be correlated, and these samples were also most likely to have wildtype p53. To determine a possible mechanism for this observed correlation, cell culture was again used to stably transfect a wildtype p53 cell line with either a control plasmid, or a plasmid with the HBV 'x' protein (*HBx*). Those cells with *HBx* showed a decrease in ASPP2 expression which could be attributed to a colocalisation of DNMTs 1 and 3A with the promoter region of ASPP2. Zhao et al are able to show that methylation of promoters allows *HBx* to control gene expression. Further work will be needed to determine whether the increased

methylation itself is a causal factor in tumourigenesis, or whether it is a side effect of another interrelated process (Zhao et al., 2010).

Alongside protein binding methods described above, the Micro-RNA (mirRNA) system has emerged as a method of regulation of cancer related genes; these mirRNAs are able to effectively regulate oncoprotein expression (He et al., 2007). MirRNAs are double stranded RNA molecules which are transcribed, like protein coding genes, by RNA polymerase II (Thakur, 2004). Although first discovered as an essential component of larval timing in *C.elegans*, mirRNAs are now known to be important in proliferation, differentiation, apoptosis and development in metazoans, and thus have the potential to control cancer development. Recently, it has been reported that the mirRNA-34 family are direct targets of p53 binding, thus raising the possibility that they are able to mediate the effects of the apoptosis and cellular senescence pathways (He et al., 2007). They function by simultaneously controlling the expression of a wide range of targets, thus it has been difficult to understand their exact role in tumour suppression. A recent paper by He *et al* aimed to determine the function of mirRNA-34s in a specific tumour example; osteosarcomas (He et al., 2009). Osteosarcoma is interesting to study, due to its low mutation rate of p53. On average only 15% of these cancers have a loss of function or knock out of p53, thus another factor must be causing a majority of the changes in regulation which lead to tumourigenesis (Petitjean et al., 2007). It is possible that mutation or other changes in mirRNA-34 will prevent the transduction of the apoptosis or cellular senescence signal which has been initiated by p53. Initially the study used two cell lines, one with Wildtype p53 and one with a loss of function mutation. They showed that in response to genotoxic stresses, p53 is essential to bring about an upregulation of mirRNA-34. A more interesting finding, however, was the fact that artificial upregulation of mirRNA-34 using synthetic RNA mimic molecules was naturally amplified in cells containing wildtype p53. This suggested that although

Mir34 was downstream of p53, a more complex regulation system existed to control the specific level of mirRNA-34 being produced.

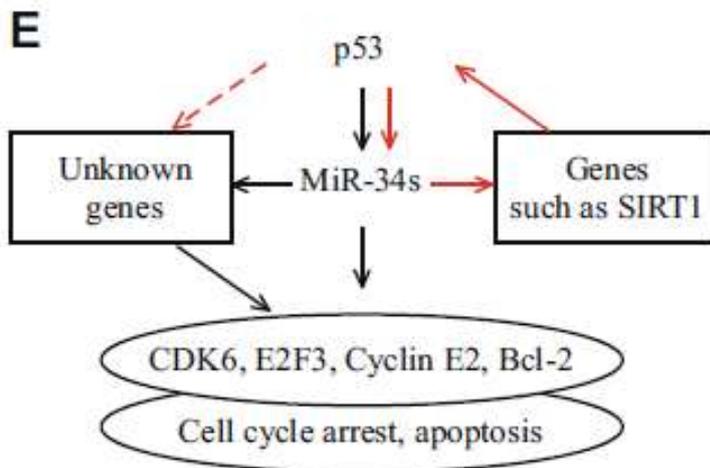


Figure 1 A feedback loop is likely to exist between p53 and its downstream microRNA target. Recently identified genes such as SIRT1 are possible candidates for this process. In addition, it is likely that a second level of regulation exists via as yet un-identified genes; this would allow p53 to exert control over the tumour suppression pathway with or without microRNAs (He et al., 2009).

There are two complimentary models which could explain this (see Figure 1). Firstly, although MiR34s have been identified as binding targets of cell-cycle and apoptosis controlling genes, it is possible that MiR34 also has an effect via secondary genes, which could also be controlled by p53. This would create a secondary level of regulation and a level of redundancy in the system, which could be crucial in such an important pathway. Secondly, a feedback loop could exist from mirRNA-34, which would require p53 to fully amplify the response. After characterising the phenotypic effects of MiR-34 in cell culture, 117 primary samples were taken from osteosarcoma patients in a paired fashion; one sample from tumour tissue and one from adjacent normal tissue. MiR-34 level was measured using RT-PCR, and tumour tissue was shown to have almost a 50% decrease in expression in comparison to normal tissue. To determine the cause of this decrease, high throughput sequencing was carried out; only 7/117 samples contained a mutation which could cause a decrease

in function. This suggested that another mechanism could have been responsible for the changes observed. Methylation was proposed as a possible explanation. High resolution melting is a process which can determine very small changes in nucleotide structure and modification through measuring the temperature at which two strands of DNA separate. Using this technique, it was shown that over 50% of the tumour samples had a significantly higher level of methylation than adjacent normal tissue; this provides a mechanism in which MiR-34 downregulation could occur. As the previous characterisations had shown, a down regulation of MiR-34 is enough to cause a significant decrease in p53 mediated apoptosis and cellular senescence (He et al., 2009).

The two studies which I have chosen to highlight are prime examples of a new approach to studying oncoprotein regulation; although work such as the human genome project have increased our understanding of genetic problems, it only begins to identify factors outside of DNA sequences. Epigenetic factors such as Methylation and histone modifications have caused a paradigm shift cancer biology; they hold the possibility of answering a number of difficult questions. It is likely, however, that initially they will complicate research even further.

Words: 1487

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