Commentary

Acetyltransferases and tumour suppression

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Abstract

The acetyltransferase p300 was first identified associated with the adenoviral transforming protein E1A, suggesting a potential role for p300 in the regulation of cell proliferation. Direct evidence demonstrating a role for p300 in human tumours was lacking until the recently publication by Gaythe*r et al*, which strongly supports a role for p300 as a tumour suppressor. The authors identify truncating mutations associated with the loss or mutation of the second allele in both tumour samples and cell lines, suggesting that loss of p300 may play a role in the development of a subset of human cancers.

Keywords: acetylation, acetyltransferases, CBP, E1A, p300, tumour suppressors

Post-translational modifications have long been recognised as important in the regulation of protein function and, until recently, phosphorylation has received the most interest and attention. Over the past few years, however, an appreciation of the role of protein acetylation has increased rapidly, with suggestions that it may rival phosphorylation as a critical regulatory modification [1]. CBP and p300 are two closely related proteins that function to acetylate target proteins, and they can form interactions with a growing list of cellular proteins, including transcription factors that play important roles in the regulation of cell growth, such as c-jun, E2F-1, p53, and NF-κB [2]. Although CBP and p300 have been postulated to function as tumour suppressors, unequivocal evidence from human malignancies has been lacking. A recent report by Gaythe*r et al* [3] takes a significant step in rectifying this deficiency, with the identification of truncating mutations of p300 being associated with mutation of the second allele in both tumour samples and cell lines.

Both CBP and p300 are histone acetyltransferases (HATs) [4,5], as are a number of other transcriptional coactivators, including TAF250 and P/CAF [6,7]. One mechanism through which these HATs are thought to regulate transcription is the modulation of chromatin structure, via acetylation of histones, which relaxes the interaction of histones with DNA. The acetylases can form a bridge between transcription factors and the basal transcription machinery [2], therefore enhancing transcriptional activation. Since CBP and p300 are present at limiting concentrations within cells, competition for them between different transcription factors can facilitate integration of different signal transduction pathways [8,9]. Although the acetylation of histones is an important property of CBP and p300, in terms of mediating transcriptional activation, there is growing evidence for the importance of acetylation of other protein targets [1]. These other substrates for CBP and p300 include components of the basal transcription machinery and transcription factors such as

HAT = histone acetyltransferase.

p53 and E2F [10,11]. The acetylation of these factors can modify their activity in either a positive or a negative manner, and, in the case of p53, this acetylation enhances sequence-specific DNA binding and transactivation. The activity of the acetylases is not limited to the transcriptional machinery, however, and the modification of nuclear import factors and cytoplasmic proteins such as α-tubulin [12] suggests that acetylation is an important regulatory modification for a wide range of cellular processes. This is shown further by the diverse effects of acetylation on protein function, including DNA binding, protein–protein interaction and protein stability. Given this broad range of possible activities for CBP and p300, it is not surprising that they are thought to play some role in the regulation of cell division and, as such, are potential candidates for oncogenes or tumour suppressors. The importance of regulation of acetylation in the control of tumour development is also highlighted by the observation that deacetylases have been found in association with tumour suppressor proteins. This is probably best illustrated by the interaction of HDAC1, HDAC2 and HDAC3 with the product of the retinoblastoma susceptibility gene (pRb), which allows pRb to function as a tumour suppressor [13].

The first suggestion that CBP and p300 may regulate growth and function as tumour suppressors was provided by studies of adenovirus E1A's transforming properties. Both CBP and p300 can interact with E1A, and this interaction perturbs the CBP/p300 activity [14]. The importance of this interaction with E1A was demonstrated by studies showing that an E1A mutant which is unable to bind to pRb but retains p300 binding was still capable of stimulating entry into DNA synthesis in rodent cells [15]. Although interpretation of this work is complicated by confusion regarding whether E1A inhibits or enhances CBP/p300 activity, the data suggest that disruption of CBP/p300 activity may stimulate proliferation, indicating that CBP/p300 may inhibit proliferation and function as tumour suppressors. Analysis of patients with Rubenstein–Taybi syndrome provided further evidence for a role for CBP in tumour suppression. Individuals with this condition lack one functional allele of CBP, display a variety of developmental syndromes and show a predisposition to cancer [16]. Although CBP and p300 are frequently discussed as interchangeable proteins, analysis of knockout mice suggests that, despite a degree of functional overlap, the two proteins can perform unique functions. Interestingly, mice heterozygous for loss of CBP develop tumours, similar to the patients with Rubenstein–Taybi syndrome, whereas mice heterozygous for loss of p300 show no increased incidence of malignancy [17].

Despite the current lack of direct evidence for p300 acting as a tumour suppressor in the mouse model, p300 itself has been implicated, by loss of heterozygosity studies, in a number of different cancer types in humans, including glioblastomas and colorectal tumours. A single case of missense mutation coupled with the loss of the other allele has also been reported for a gastric and colorectal tumour sample [18]. Although the implications of these mutations are provocative, it was unclear whether the mutations would impair the function of the resultant proteins. The mutations described by Gayther *et al* [3] could dispel these doubts because a number of them would clearly abrogate the normal function of the protein. These workers screened 193 tumour samples or cell lines, and identified six mutations that would result in the expression of a truncated protein, two of which were in primary tumours (colorectal and breast) and the other four in cell lines (colorectal, breast and pancreatic). Inactivation of the second allele was found for five out of these six samples. In addition to these truncation mutations, an insertion (in a primary breast cancer) and three missense mutations (in one primary and two colorectal cell lines) were identified; two of these were associated with deletion of the second allele. Of the truncations, three occur upstream of the HAT domain, and would clearly abrogate their normal function. The other truncations may also be inactivating despite retaining the HAT domain because they lose the ZZ/TAZ domain, which is important for interaction with a number of transcription factors, including p53 [19]. The status of the missense mutations in terms of the effects on gene function is unknown, but the insertion of six amino acids occurs in the HAT domain and may well abolish HAT activity. Most of the p300 mutations would clearly lead to loss of function, supporting the proposal that loss of p300 activity contributes to tumour development. However, the study from Gayther *et al* [3] also indicated that p300 mutations are relatively rare in tumours (10/193 samples, consisting of 6 truncations, 1 insertion and 3 missense mutations). It would be interesting to determine whether CBP is mutated in any of the tumours with wild-type p300 or whether there are other, as yet unknown, p300-related proteins that may be mutated during tumorigenesis.

The next important question to be addressed is how p300 mediates its tumour suppressive properties. One attractive hypothesis would be that, since p300 is involved in enhancing the transcriptional activity of p53 [10,19–21], loss of its function would impair the ability of p53 to function as a tumour suppressor. However, a p53-independent tumour suppressor function for p300 is suggested by studies showing that the proliferative advantage of perturbation of p300 by E1A occurs even in the absence of functional p53 [15]. Gayther *et al* addressed this question in the human cancer cells by testing the samples for the presence of p53 mutations. Three of the six samples with mutations leading to truncations of p300 contained p53 mutation, indicating that loss of p300 does not eliminate the need for loss of p53 during tumour development. Nevertheless, a role for p53 is not completely eliminated; it is possible that loss of p300 impairs p53 function only

partially and, despite giving the cell a selective advantage, there would still be selective pressure to lose p53 function completely. Further work is clearly required to understand how loss of p300 may contribute to tumour development, but there is an abundance of other target proteins with which it can interact that may mediate this phenotype.

Gayther *et al* [3] strongly support a role for p300 as a tumour suppressor, and reveal an interesting dual role for CBP/p300 in the development of human cancer. In addition to functioning as tumour suppressors, both CBP and p300 can, in certain leukaemia strains, be oncogenic as a result of fusion to other proteins [2,22,23]. In this case, these mutations are of gain-of-function type, which presumably function by inappropriately enhancing activation of certain transcription programmes leading to increased proliferation. Identification of the protein substrates of p300 and CBP that mediate the positive or negative regulation of cell growth is complicated by the difficulty of assigning which acetylase *in vivo* is responsible for the acetylation of a protein. However, the recent generation of specific synthetic inhibitors of acetylases [24] may allow the identification of pathways regulated specifically by p300 and CBP, and may shed light on how they function as tumour suppressors.

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