Minireview

A role for Numb in p53 stabilization

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Abstract

The cell-fate determinant Numb has recently been shown to help activate the tumor suppressor protein p53. Loss of Numb in breast cancers would result, therefore, in both the activation of the potential oncogene Notch and the diminution of tumor suppression by p53.

The tumor suppressor protein p53 acts primarily as a transcription factor, inducing the expression of genes involved in cell-cycle arrest, senescence and apoptosis in response to cellular stresses [1]. While these growth-inhibitory activities of p53 are important in preventing tumor development, they are severely detrimental to normal growth and development. In unstressed cells, therefore, p53 is kept inactive, mainly through polyubiquitination by the E3 ubiquitin ligase MDM2 and subsequent proteasomal degradation [2-4]. Loss of MDM2 binding or inhibition of its E3 ligase activity allows p53 to be rapidly stabilized and activated in response to a variety of cellular stresses that are associated with tumor development and progression, including DNA damage, oncogene activation, hypoxia and metabolic stress. The efficiency of p53 as a tumor suppressor is reflected by the perturbation of the normal p53 pathway in most, if not all, cancers. This is frequently achieved by mutations in p53 [5] or, less commonly, by amplification of the gene for MDM2 [6]. Other mechanisms to prevent the activation of p53 are also exploited by tumor cells, however, such as loss of expression of the $p14^{ARF}$, a protein that can inhibit MDM2-mediated p53 ubiquitination in response to oncogene activation [7].

It is less clear how the p53 response is by-passed in breast cancers, as mutations in p53, or changes in the expression level of MDM2 or $p14^{ARF}$ occur less frequently than in other tumors. Previous reports have suggested that in some breast cancers p53 is abnormally localized to the cytoplasm [8], thereby preventing its ability to regulate gene expression. Downregulation of the expression of ASPP - a protein that is required for p53 to induce apoptosis - has also been shown to be a common event in breast cancers [9]. A recent study by Colaluca *et al.* [10] now shows that the cell-fate determinant Numb, which is frequently lost in breast tumors, also plays an important role in the activation of p53. Until now, Numb has most commonly been associated with negative regulation of signaling from the protein Notch, a potential oncogene [11]. Loss of Numb may, therefore, be a critical step in the development of breast tumors by leading to both the activation of an oncogene and the inhibition of the p53 tumor suppressor pathway.

Numb and its partners within the cell

The cell-surface receptor Notch is involved in the regulation of cell-fate specification and may control the balance between proliferation and differentiation in development and homeostasis. Notch binds to a family of transmembrane ligands, resulting in cleavage of the receptor, translocation of the intracellular domain to the nucleus and activation of a number of target genes [12]. During embryogenesis Numb functions as an inhibitor of Notch signaling and is involved in the cell-fate decisions of a number of cell lineages. Numb is also expressed in many adult tissues, where it may have additional functions, such as E-cadherin localization [13] and suppression of signaling through the Hedgehog pathway [14].

As might be predicted from its role in regulating cell fate, self-renewal and differentiation, there are some provocative links between Numb and tumor development. Until now, these had been interpreted in terms of enhanced Notch signaling [15],

but a study published recently in *Nature* by Colaluca *et al*. [10] provides an extremely interesting alternative by describing a role for Numb in the regulation of p53 activity [10].

Previous studies had shown that Numb can bind to and be ubiquitinated by MDM2 [16,17], although until now the function of this interaction was unknown. Colaluca *et al*. [10] now show that Numb can actually interact *in vivo* with endogenous MDM2 and p53, resulting in a trimeric complex between the three proteins [10]. This interaction appears to regulate the stability of p53, as reduction of Numb levels by RNA interference (RNAi) causes a decrease in the half-life of p53 and consequently a reduction in steady-state levels of the protein. Consistent with this observation, overexpression of Numb increases the level of p53 in both unstressed and stressed cells. Colaluca *et al*. also investigated the effects of treating cells with genotoxic drugs, and found that these were influenced by the level of Numb. In cells in which Numb had been knocked down by RNAi, threefold higher doses of cisplatin were required to induce p53 to a similar level as in wild-type cells. This effect was also reflected in the higher levels of cisplatin required to induce expression of a number of p53 target genes. In addition, more cisplatin-induced DNA damage was observed after Numb knockdown than in wild-type cells.

Interestingly, the effect of Numb on p53 stability was shown to be dependent on MDM2. Colaluca *et al*. [10] show that Numb functions by inhibiting the E3 ubiquitin ligase activity of MDM2 towards p53, although the previous observation that Numb can be ubiquitinated by MDM2 suggests that the effect on p53 may not result from a complete loss of MDM2's E3 activity, but instead may be specific for p53. How Numb functions to prevent the ubiquitination of p53 by MDM2 currently remains unclear. It is possible that Numb simply inhibits binding of MDM2 to p53 . The authors show that the interaction between Numb and MDM2, or between Numb and p53, is not prevented by an inhibitor of the p53-MDM2 interaction, and although they demonstrate the existence of a trimeric p53-MDM2-Numb complex, it seems quite possible that the contact between p53 and MDM2 within this complex is perturbed. Alternatively, Numb may simply intercalate between p53 and MDM2, preventing ubiquitination of p53. The interaction between p53 and MDM2 has recently been shown to be more complex than initially thought. The well characterized binding between the amino termini of the two proteins causes a conformational change, which allows binding between the DNA-binding domain of p53 and the acidic domain of MDM2 [18,19]. It is now thought that it may be this second stage of binding that is critical for ubiquitination of p53, and it is possible, therefore, that Numb inhibits this second binding step without perturbing the initial p53-MDM2 interaction.

In this context, a number of other proteins - such as $p14^{ARF}$ [20,21] and several ribosomal proteins [22] - have been shown to bind to MDM2 and inhibit its E3 ligase activity towards p53 without obviously preventing the p53-MDM2 interaction. It is interesting that inhibition of the E3 ligase activity of MDM2 by proteins such as Numb can play such an important role in the regulation of p53, an observation that is consistent with studies in MDM2 knock-in mice [23]. This has revealed that binding of MDM2 to p53 may not be sufficient to switch off p53's transcriptional activity. Instead it is the E3 ligase ability of MDM2 that appears to be critical in the regulation of p53.

Intriguingly, in the case of Numb it seems unclear whether it is the interaction with MDM2 or the interaction with p53 that is key to protecting p53 from ubiquitination by MDM2. Indeed, Colaluca *et al*. [10] show that p53-stabilizing signals, such as treatment with the protein Nutlin, an inhibitor of the p53-MDM2 interaction, or the drug cisplatin, strongly decreased the Numb-MDM2 interaction but not that of Numb and p53. As they show that Numb is important for the efficient stabilization of p53 in response to cisplatin, the results suggest that the critical interaction may be the one between Numb and p53.

At least two mechanisms by which Numb may function have been established in previous studies. Firstly, Numb has been shown to interact with various components of the endocytotic machinery and can affect the endocytosis of Notch (as well as other proteins) [24-26]. How such an activity might relate to the modulation of the relationship between p53 and MDM2 - both nuclear proteins - is less than obvious. However, Numb has also been shown to influence ubiquitination of proteins such as Notch [27] or the Hedgehog transcription factor Gli1 [14] - either directly or through interaction with the ubiquitin ligase Itch. It is interesting to note that rather than inhibiting ubiquitination, Numb can enhance the degradation of Gli1 by Itch - again pointing to a rather specific effect of Numb on the stability of p53. Rather provocatively, the p53-related protein p73 is a target for ubiquitination and degradation by Itch [28]: is it possible that Numb plays a much deeper role in controlling the whole p53 family?

Numb and p53 in normal and cancer cells

The identification of Numb as a factor controlling p53 activity leads obviously to the possibility that Numb can regulate cancer development. Loss of Numb occurs frequently in breast tumors, leading to activation of oncogenic Notch signaling [15] and, as now found by Colaluca *et al*. [10], inactivation of the p53 tumor suppressor pathway. These authors also compared primary tumor cells with low or normal levels of Numb, revealing that loss of Numb correlates with reduced steady-state levels of p53 and also with resistance to chemotherapeutic drugs, more aggressive neoplastic disease and poor prognosis [10]. As Colaluca *et al*. show that inhibition of MDM2 effectively stabilized p53 -

even in Numb-deficient cells - these results suggest that small molecules directly targeting MDM2 might be effective both in cancers overexpressing MDM2 and cancers deficient in Numb.

Finally, the appearance of p53 in the Numb pathway leads to some interesting speculation about why and when Numb might regulate p53 during normal growth and development. There is no clear evidence that Numb expression or activity is induced by oncogenic stress; instead, Numb activity is regulated by asymmetric partitioning at mitosis, leading to unequal distribution of Numb in daughter cells that are then destined for different fates [29]. Is it possible that differential regulation of p53 in the two daughters contributes to this choice of fate - or that deregulation of this level of control by loss of Numb results in a symmetric division of breast stem cells, resulting in abnormal and ultimately malignant growth? It is doubtful that the p53 field will remain numb to these possibilities for long.

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