C/EBP δ : friend or foe? A novel role for C/EBP δ in metastasis

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Hypoxia contributes to major aspects of cancer biology such as angiogenesis, glucose/energy metabolism, invasion and metastasis. The activation of hypoxia-inducible factor 1 α (HIF-1 α) is a key step that promotes the transcriptional activation of target genes fundamental for these processes. In this issue of *EMBO Journal*, Balamurugan *et al* provide new insights on how hypoxia control integrates with inflammation and metastasis by describing an exciting role for the transcription factor C/EBP δ in the amplification of HIF-1 α signalling. The authors show that C/EBP δ promotes the transcriptional repression of the tumour suppressor F-box and WD repeat-domain containing 7 protein (FBXW7), which in turn leads to mTOR protein stabilization and increased HIF-1 α levels. Although C/EBP δ can act as a tumour suppressor gene at early stages of cancer development, it appears to have a positive role in progression and metastasis, thus adding to the ranks of genes that exert opposing effects at early and late stages of tumorigenesis.

An increasing body of evidence suggests that C/EBP δ functions as a tumour suppressor (Pawar *et al*, 2010; Wang *et al*, 2010). In this issue of *EMBO Journal*, Balamurugan *et al* provide *in vivo* evidence that germline deletion of this gene promotes increased tumour number in transgenic mice overexpressing the Neu/Her2/ERBB2 proto-oncogene in the mammary gland. Surprisingly, the authors report that though an increased number of primary tumours was observed in C/EBP δ -null mice, a paradoxical two-fold reduction in the incidence of metastasis was seen in the same animals. After some careful detective work, Balamurugan *et al* came to the conclusion that the mechanism involves an intricate interplay between C/EBP δ and HIF-1 α . First, a link between hypoxia and C/EBP δ was established by the discovery of high immunoreactivity against C/EBP δ specifically in hypoxic areas of mammary tumours. Using isolated mammary tumour cells, the authors then showed that, while in WT cells HIF-1 α is strongly induced under hypoxic culture conditions, cells derived from C/EBP δ -null tumours fail to show such induction. Fast growing tumours outpace the ability of vasculature to deliver enough oxygen and nutrients. HIF-1 α activation is critical for adaptation of cells to hypoxic stress, in part, by promoting transcriptional changes leading to anaerobic metabolism (Semenza, 2010). C/EBP δ -null tumour cells fail to implement this adaptation as shown by decreased viability, glucose uptake and lactate production when subjected

to hypoxia. These results seem to confirm a fundamental role of C/EBP δ in the establishment of a successful adjustment to low oxygen levels.

The serine-threonine protein kinase mTOR serves as a central regulator of cell metabolism, growth, proliferation and survival (Laplane and Sabatini, 2009). Oncogenic activation of this pathway by loss of function of several tumour suppressors (LKB1, PML, PTEN, TSC1/TSC2) or

Figure 1 (A) C/EBP δ expression is restricted to hypoxic tumour areas and increases mTOR stability through repression of FBXW7. C/EBP δ acts as a tumour suppressor through various mechanisms leading to decreased primary tumour formation. Balamurugan *et al* now propose that C/EBP δ acts through HIF-1 α stabilization to promote metastasis formation. This effect is dependent on repression of FBXW7, which leads to increased mTOR and HIF-1 α . (B) C/EBP δ parallels TGF β effects on tumorigenesis. C/EBP δ has also been shown by Bengoechea-Alonso *et al* to influence TGF β signalling, although in the latter case the directionality of the effect in this context is unclear. FBXW7 also controls levels of CEBP α , and may mediate crosstalk between these transcription factors.

gain of function affecting protein tyrosine kinases, Ras and/or the downstream phosphatidylinositol-3-kinase/AKT pathway increases the rate of translation of HIF-1 α mRNA into protein (Semenza, 2010). Using mouse embryonic fibroblasts, Balamurugan *et al* provide evidence to suggest that C/EBP δ increases HIF-1 α induction by promoting its translation. Accordingly, the authors show that cells deficient in C/EBP δ have reduced activity of the phosphatidylinositol-3-kinase/AKT/mTOR pathway as shown by decreased basal and serum-induced AKT and S6K1 phosphorylation. These alterations are accompanied by decreased mTOR stability leading to defective HIF-1 α activation, which can be rescued by exogenous C/EBP δ expression. Interestingly, in the absence of C/EBP δ , mTOR showed increased polyubiquitination and its reduced stability could be reversed by proteasome inhibition, leading to the conclusion that reduced mTOR levels in C/EBP δ -null cells are due to degradation by the ubiquitin-proteasome system.

A further piece of this complex puzzle was the observation that C/EBP δ directly inhibits transcription of the FBXW7 gene. FBXW7 is a member of the F-box protein family and functions as a component of the SCF (SKP1-cullin-F-box) ubiquitin protein ligase complex, and a potent haploinsufficient tumour suppressor (Mao *et al*, 2004). Among the important oncoproteins controlled by FBXW7 at the level of protein stability are cMyc, Notch, cJun, and, importantly for this discussion, mTOR (Mao *et al*, 2008; Welcker and Clurman, 2008). Balamurugan *et al* conclude that C/EBP δ , by inhibiting FBXW7 and causing increased stability of mTOR in the tumour cells, leads to an increase in HIF-1 α protein levels, contributing to tumour progression and metastasis (Figure 1A).

As with many interesting studies, however, the conclusions raise additional questions that remain to be addressed. Although the authors favour a cell-autonomous action of C/EBP δ in the mammary tumours, non-cell-autonomous effects in the stromal cells and tumour microenvironment cannot be ruled out, particularly as they used mice carrying a germline deletion of C/EBP δ . As the biological activity of FBXW7 is strongly dosage dependent, subtle changes in levels caused by loss of CEBP δ in multiple cell types may well contribute to the phenotypes observed. It is also difficult at this stage to reconcile the results of Balamurugan *et al* with published data on the significance of C/EBP δ in human oncogenesis. Chiefly, its inclusion in a 70-gene signature predicting better survival of breast cancer patients

(Naderi *et al*, 2007) does not readily fit with its proposed pro-metastatic function. Again, the use of a global knockout strategy might obscure the effects of somatic loss of C/EBP δ during tumour progression and metastasis. In this regard, further studies using tissue specific and/or temporally controlled targeting could provide new and valuable insights. The finding that an early inflammatory response gene such as C/EBP δ amplifies HIF-1 α induction begs the question of what is the role of this mechanism in immune-mediated responses to tumour cells. Recent data (Corzo *et al*, 2010; Doedens *et al*, 2010) suggesting a prominent role for HIF-1 α in tumour-associated macrophage biology raise exciting questions about the potential role of C/EBP δ in these processes.

In the light of these new data, C/EBP δ joins the ranks of other genes with a dual role in tumorigenesis, the paradigmatic example being TGF β . Indeed, the parallels between these biological pathways are quite striking (Figure 1B). The tumour suppressor activity of both proteins has been ascribed to their ability to cause growth arrest, induce apoptosis and control genomic stability. Both have suppressor effects on early stage tumour development, but can promote progression and metastasis. The mechanisms used by TGF β to promote metastasis have been intensively investigated and include both cell-autonomous effects in induction of the epithelial mesenchymal transition leading to an invasive metastatic phenotype, as well as non-cell-autonomous effects on the immune system and tumour microenvironment (Barcellos-Hoff and Akhurst, 2009). It remains to be seen whether such a disparate range of mechanisms is also employed by C/EBP δ to influence tumour progression. Tantalizing links between FBXW7 and both TGF β and CEBP α signalling (Bengoechea-Alonso and Ericsson, 2010a,b) provide a hint of the complexity of the interacting networks that endow cancer cells with their seemingly endless flexibility in discovering new routes to progression.

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Conflict of interest

The authors declare that they have no conflict of interest.

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