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Commentary

E2F1 selects tumour cells for both life and death

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Abstract

E2F1 is a transcription factor involved in both cell cycle progression and apoptosis. Perhaps surprisingly, these two processes are closely related, and the choice between them appears to be made on the basis of the aggregate of signals flowing into the cell at the time. This may be the means whereby normal cells tune their threshold for apoptosis with respect to the availability of external growth factors, so that cells that are supernumerary to the tissue's needs at the time can be immediately deleted. In many tumours, however, the pathways that link E2F1 activity to apoptosis have been interrupted, sometimes at multiple points. Non-small-cell lung carcinoma provides a striking example of this, with the result that expression of E2F1 in these tumours does not correlate with apoptosis but is a good surrogate marker for replicative status. This relationship does not necessarily pertain in other tumour types. Molecules such as E2F1 lie at the core of very significant cell fate decisions, but they are part of a complex matrix of interactions, all of which must be surveyed before interpretation in terms of tumour behaviour is possible. Microarray analysis may provide a way to do this. In the future, however, such interpretations, including predictions of therapeutic response, may be possible through interrogation of the status of a relatively limited number of molecules. Those that preside over critical cellular decision forks (such as the choice between proliferation or death) are good candidates for this role. E2F1 clearly qualifies as one member of this group.

Keywords: **E2F1; cell cycle; apoptosis; G1/S phase**

In this issue, Gorgoulis and his colleagues describe the expression of the transcription factor E2F1 in a large series of non-small-cell carcinomas of lung [1]. They show that E2F1 is expressed in a higher proportion of cancer cells than in the non-neoplastic adjacent respiratory epithelium, and that this is usually attributable to increased transcription rather than modification of stability of the E2F1 protein alone. In a small number of tumours, the E2F1 gene was amplified, and in one there was an apparently tumour-specific mis-sense mutation. Why should these findings be of interest?

E2F1 was first identified as a cellular factor required for induction of the adenoviral early gene E2 by the viral transforming gene E1A – and hence its name. It is an essential part of the circuitry that commits cells to progression through the G_1 phase of the cell cycle and into DNA synthesis (reviewed in ref [2]). In particular, it pilots cells through the 'restriction point' in G_1, the point early in cell cycle progression at which commitment is made to traverse the entire cycle, including replication of genomic DNA, the subsequent partition of chromatids at mitosis, and the ultimate separation at cytokinesis into two daughter cells. Cells in which E2F1 (together with its close homologues E2F2 and E2F3) have been silenced by conditional mutations cannot make this commitment and fail to proliferate [3]. Under normal circumstances, in contrast, cells that have passed the restriction point require no further external stimuli (for example from growth-factors in their microenvironment) in order to complete the proliferative cycle, although such progression may still be halted by cell injury. A good deal is known of the mechanism involved in this critical action of E2F1, and much of it is relevant to carcinogenesis.

Movement from early G_1 towards the restriction point depends upon phosphorylation of the oncosuppressor protein Rb, and is effected by the formation and activation of a nucleoprotein complex involving cyclin D1 and its dependent kinases, cdk4 and cdk6 (Figure 1). In normal cells, progression around the cycle is dependent on external growth factors (such as EGF, FGF, PDGF and the insulin-dependent growth factors IGF-1 and IGF-2) because such factors facilitate the formation and activation of these cyclin/kinase complexes. Their action is opposed and regulated by a group of low molecular weight inhibitors such as $p16^{INK4A}$ and p19, themselves under regulation from cytokines such as TGFβ. E2F1 forms a complex with unphosphorylated Rb, lying within a pocket formed by the C-terminal domain of the Rb molecule. The outstanding result of Rb phosphorylation is release of E2F1 from this pocket. This exposes the transactivation domain of E2F1, which then initiates transcription of a large but precisely determined set of genes [4]. Amongst these are several whose products are required for DNA synthesis (eg thymidine kinase, dihydrofolate reductase, DNA polymerase α, PCNA, CDC6 and the pre-replicative chromatin proteins ORC1 and MCM2-7). Others include the genes encoding E2F1 and E2F2 themselves, suggesting the existence of a positive

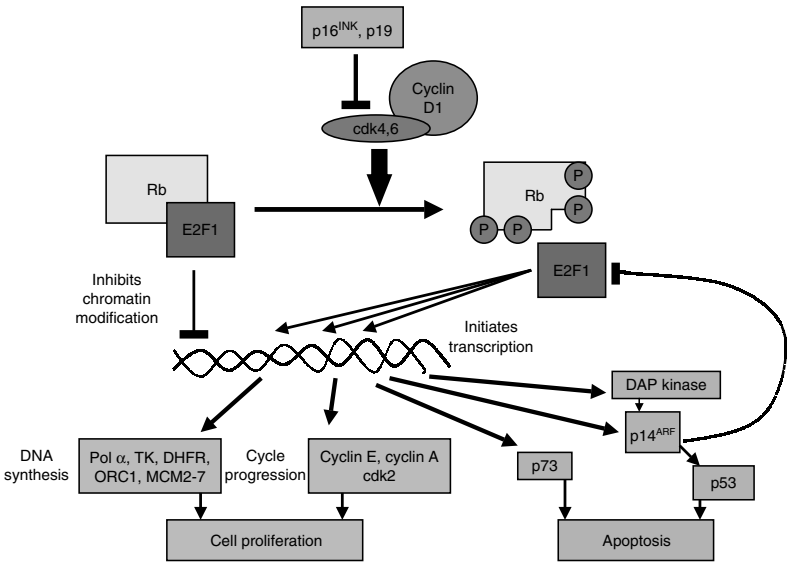

Figure 1. E2F1, activated as a result of phosphorylation of the retinoblastoma protein (Rb), initiates transcription of genes that determine progression around the cell cycle and others that activate apoptosis. In non-small-cell lung cancer, but not necessarily other tumours, the pro-apoptotic effects are eroded by several apparently independent mechanisms, including gene methylation, gene loss, and inactivating mutations. Because $p14^{ARF}$ normally inhibits E2F1 expression, these changes may result in both an increase in E2F1 expression and loss of the apoptotic functions of E2F1, whilst the cell replicative functions are retained. Pol α: DNA polymerase α; TK: thymidine kinase; DHFR: dihydrofolate reductase; ORC: (replicative) origin binding protein; MCM: minichromosome maintenance protein; cdk: cyclin dependent kinase

feedback loop, capable of amplifying the effect of E2F activation. Still others encode proteins essential for cell cycle progression, such as cyclins E and A, and cdk1, 2 and 4. This has much to do with the role of E2F1 in facilitating transit of the restriction point, which appears to be synonymous with activation of the cyclin E-cdk 2 complex and movement into late G1.

The activation of E2F1 through phosphorylation of Rb is thus a crucial event permitting movement through the cell cycle. Recent data also show that the complex of unphosphorylated Rb and E2F1 has important and complementary functions of its own, as it recruits histone deacetylase and so inhibits the chromatin remodelling required in the preparation for changes in transcription. For these two reasons, therefore, the intranuclear appearance of E2F1, together with loss of unphosphorylated Rb, signals that movement around the cell cycle is free to proceed. Persistence of this 'chocks away!' signal, regardless of the cell environment, appears to be an almost universal feature of the tumour phenotype. Thus, in tumours of many different types, the Rb gene is commonly mutated or deleted, but in those tumours with intact Rb there is usually some other abnormality of the Rb-E2F pathway. Thus cyclin D1 is often mutated to a constitutively active state, or there may be functional deficiency in the p16/p19 cdk inhibitors, often a result of failed TGFβ signalling. In the ship launch analogy, cells with any of these abnormalities do not wait for the fractured champagne bottle (ie stimulation by specific environmental signals), but are in a constant state of charging down the slipway of the cell cycle.

In their lung carcinoma series [1], Gorgoulis *et al.* observed that those tumours with raised nuclear E2F1 demonstrated a higher proliferation index (as judged by Ki67 immunocytochemistry) than those without. There was also a positive correlation between E2F1 expression and deficient nuclear staining for Rb. Against the background of the biology of E2F1 and Rb described above, these associations might seem hardly controversial. The story of E2F1 has another side to it, however. The enforced expression of E2F1 in cultured cells has repeatedly been shown to cause apoptosis [5–7]. This is partly due to transactivation of the protein $p14^{ARF}$ by E2F1, either directly or through activation of the death associated protein (DAP) kinase [8]. $p14^{ARF}$ is encoded from an alternatively spliced transcript of the $p16^{INK4A}$ gene. It stabilises p53 by displacing it from hMDM2, the p53-binding protein that normally ensures swift degradation of p53 by the proteasome pathway. There are also p53-independent routes whereby E2F1 can stimulate apoptosis: activation of the p53 homologue p73, inhibition of NFκB [9] and down-regulation of Mcl-1, an antiapoptotic Bcl2 family member [10] are known examples. E2F1 thus signals proliferation and apoptosis simultaneously. This appears to be part of a fundamental regulatory mechanism that lowers the threshold for apoptosis in cells during proliferation. This non-intuitive mechanism may serve to protect tissues from the undesirable consequences of DNA replication, one of which is carcinogenesis. It was first identified a decade ago [11] when it was shown that c-*myc*, an oncogene required to sustain the cell cycle, also activates apoptosis. It has now been shown that c-*myc*-induced apoptosis is indeed mediated through E2F1 [12]. In this way, E2F1 can function as an oncosuppressor, and there are studies that show reduction

of E2F1 expression in breast carcinoma [13] — the opposite effect to that described by Gorgoulis *et al*. in non-small-cell carcinoma of lung [1].

So can the differing levels of E2F1 expression in different tumour types be explained and reconciled, and will they ever they help us understand, diagnose or treat tumours? Hopefully the answer to both these questions is yes. Initiation of apoptosis and replication are closely intertwined, but which is finally selected and completed is determined by the aggregate of signals flowing into the cell at the time. As Figure 1 seeks to show, the pathways between E2F1 and apoptosis are multiple and interactive rather than simple and linear. Although this is sometimes referred to as 'redundancy', the profusion of parallel pathways within signalling networks is far from unnecessary. Under normal circumstances, it permits activities affecting one pathway to amplify or diminish the effects of others. In this way, the triggering of major and irreversible events (such as cell proliferation and cell death) can be tuned to multiple parameters that define the prevailing status of the cell. In tumours, gene suppression by methylation, mutation or haplo-insufficiency can produce caricatures of this tuning, permanently blocking one outcome such as apoptosis in favour of replication and survival. In non-small-cell lung carcinomas, for example, there are frequently multiple deficiencies in the pathways that lead from E2F1 to apoptosis. The DAP kinase gene is often inactivated through hypermethylation [14], p53 may be mutated, and both the $p16^{INK4A}$/$p14^{ARF}$ locus itself [15,16] and p73 [16] frequently show haplo-insufficiency. Moreover, a negative feedback loop has recently been described in human cells whereby $p14^{ARF}$ suppresses transcription of E2F1 [17]. Non-small-cell lung cancers, with deficient $p14^{ARF}$, may not be able to effect this down-regulation, so the proliferative responses to E2F1 could be intensified, whilst the pro-apoptotic are suppressed. In other types of tumour cell, however, the expression of E2F1 may lower the threshold for apoptosis, making such cells more sensitive to chemotherapeutic agents [18]. Thus, examination of single elements of the molecular network that regulates proliferation and apoptosis may produce conflicting results when one tumour is compared with another. With the advent of expression microarray technology, however, it is now possible to assess the status of the entire network. There is real hope that such data will lead to precise predictions, not only of the overall likelihood of proliferation or apoptosis, but of the effects on these of specific therapeutic modalities.

Microarray data that describe the global effects of E2F1 and E2F2 activation are already accumulating [4]. They confirm that the E2F transcription factors induce the proteins associated with cell cycle progression from G_1 to S phase, as described above. Interestingly, they also suggest that there is a group of E2F-dependent proteins that may appear much later in the cell cycle — the repair protein RAD51, the mitotic initiator cyclin B and the kinetochore protein BUB1. Dysfunction of such proteins is capable of producing another characteristic tumour phenotype — aneuploidy.

These studies have provided a glimpse into a regulatory network of great complexity, with correspondingly subtle patterns of disorder within tumours. Yet the fact that well-known molecules re-appear with different roles in tumours of different types suggests that the number of elements in these patterns will prove to be limited: indeed we may not be far from knowing something about most of them already. For sure, E2F1 is amongst them.

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