

E4F1 dysfunction results in autophagic cell death in myeloid leukemic cells

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Abbreviations: AML, acute myeloid leukemia; HS, histiocytic sarcoma; HSC, hematopoietic stem cell; KO, knockout; ROS, reactive oxygen species; ER, estrogen receptor; 4OHT, 4 hydroxy-tamoxifen.

Summary

The multifunctional E4F1 protein was originally identified as a cellular target of the E1A adenoviral oncoprotein. Although E4F1 is implicated in several key oncogenic pathways, its roles in tumorigenesis remain unclear. Using a genetically engineered mouse model of myeloid leukemia (histiocytic sarcomas, HS) based on the genetic inactivation of the tumor suppressor *Ink4a/Arf* locus, we have recently unraveled an unsuspected function of *E4F1* in the survival of leukemic cells. In vivo, genetic ablation of *E4F1* in established myeloid tumors results in tumor regression. *E4F1* inactivation results in a cascade of alterations originating from dysfunctional mitochondria that induce increased reactive oxygen species (ROS) levels and ends in massive autophagic cell death in HS transformed, but not normal myeloid cells. E4F1 depletion also induces cell death in various human myeloid leukemic cell lines, including acute myeloid leukemic (AML) cell lines. Interestingly, the E4F1 protein is overexpressed in a large proportion of human AML samples. These data provide new insights into E4F1-associated survival functions implicated in tumorigenesis and could open the path for new therapeutic strategies.

E4F1 was first described as a cellular target of the viral oncoprotein E1A during adenoviral (serotype V) infection. E4F1 is a ubiquitously expressed multifunctional protein exhibiting intrinsic transcriptional and DNA-binding activities, and an atypical ubiquitin E3-ligase activity directed against the tumor suppressor protein p53. In addition, E4F1 also interacts with the retinoblastoma protein pRb, suggesting it is an important component of these two gatekeeper tumor suppressor pathways that are commonly altered during tumorigenesis. However, genetic data support the notion that E4F1 cellular functions extend beyond these 2 essential oncogenic pathways, leading us to address the unknown p53- and pRb-independent functions of E4F1 during tumorigenesis. To answer this question, we used a genetically engineered mouse model based on the *Ink4a/Arf* null and *E4F1* conditional knockout (KO) alleles. To circumvent the potential defects associated with *E4F1* inactivation in the whole organism, we generated a hematopoietic-specific tumor prone mouse model by transplanting hematopoietic stem cells (HSC) harvested from *Ink4a/Arf* KO; *E4F1* flox, *Cre-ER* compound mice into lethally irradiated wild-type recipient animals. Using this strategy, we generated a fully penetrant mouse model of histiocytic sarcoma, a myeloid leukemia originating from the monocyte/macrophage lineage. In this model, *E4F1* could be specifically and acutely inactivated in the hematopoietic system and HS upon 4-hydroxy-tamoxifen (4OHT) administration (4OHT activates the latent Cre-ER recombinase). Our data show that *E4F1* inactivation delays tumor progression in this mouse model and induces tumor regression in established HS.

Cellular and molecular analyses of HS cell lines derived from this animal model indicated that *E4F1* dysfunction induces mitochondrial defects that lead to overproduction of superoxide anions (ROS) followed by autophagic cell death. This autophagic response is abolished in the presence of ROS scavengers or activators of cellular anti-oxidant defenses, indicating that these ROS are the cause of cell death rather than an indirect consequence of

the loss of viability. Importantly, similar mitochondrial defects are also observed in various human myeloid leukemic cell lines upon shRNA-mediated depletion of E4F1. These mitochondrial defects correlate with functional alterations of the electron transport chain characterized by increased consumption of O₂ but inefficient ATP production. Surprisingly, whereas these mitochondrial dysfunctions lead to massive autophagic cell death in transformed cells, they have minor consequences on normal differentiated hematopoietic cells. Although we currently do not know the exact reasons for this differential sensitivity, these results suggest that cancer cells are “addicted” to E4F1 control on mitochondria, an addiction that could reflect metabolic adaptations associated with cell transformation. Consistent with this notion, our pilot study on human AML bone marrow samples suggests that the E4F1 protein is overexpressed in leukemic cells. The metabolic alterations that render cancer cells sensitive to E4F1-depletion and that require its overexpression for survival may represent a very interesting Achilles' heel of tumor cells that could be the basis for new anti-tumor strategies.

The role(s) of ROS and autophagy during tumorigenesis remains controversial. On one hand it has been suggested that low ROS levels promote transformation by inducing genomic alterations. On the other hand, ROS can have more beneficial roles by acting as second messengers that modulate cellular pathways involved in metabolism and differentiation. In addition, high levels of ROS are also clearly associated with cell death and the pro-oxidant properties of many anticancer drugs are undoubtedly involved in their efficiency. Autophagy can also play dual roles during the process of tumorigenesis, and experimental evidence supports both tumorigenic and tumor suppressive functions of autophagy. Under hypoxic, oxidative- and/or metabolic-stress conditions, autophagy likely promotes tumor development by clearing damaged organelles and protein aggregates, thereby attenuating the immune response and providing essential metabolites required for cancer cell survival. However,

inhibition of autophagy might also promote tumorigenesis as illustrated by the increased tumor incidence observed in *beclin*-inactivated mice.

Our data identify the multifunctional E4F1 protein as a novel essential regulator of this complex mitochondria-ROS-autophagy network that is implicated in cell survival of leukemic cells with deficiencies in the p53 and pRb-pathways. Our findings are of potential therapeutic interest to develop novel anticancer treatments based on ROS-induced autophagic cell death, in particular in tumors harboring genetic alterations of the apoptotic machinery. Nevertheless, given the pleiotropic functions of E4F1 during early embryonic development and in adult tissue homeostasis, notably through its role in epidermal stem cell maintenance, there is an urgent need to identify the full “repertoire” of E4F1 targets (transcriptional targets and substrates of its E3-ligase activity) involved in mitochondrial homeostasis and metabolism in order to help in designing more subtle anticancer strategies altering some but not all E4F1-associated activities. Further work will be necessary to demonstrate that E4F1 is a real therapeutic target, but our data suggest for the first time that some E4F1-regulated metabolic activities are essential for cell survival of transformed cells.

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