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The transcriptional repressor RIP140 is a cell-cycle regulated gene which controls E2F1 activity and cell proliferation

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The transcription cofactor RIP140 (also known as NRIP1) is involved in complex transcriptional regulatory loops, acting as a repressor of several nuclear hormone receptors which positively regulate its expression at the transcriptional level (for a review, see Augereau et al., Nucl. Recept. Signal. 4:e024, 2006). Transcriptional repression by RIP140 implicates several inhibitory domains and different effectors such as HDACs and CtBPs. As demonstrated by experiments performed in mice knock-out for the RIP140 gene, this transcriptional cofactor is essential for female fertility and energy homeostasis.

In this study, we have evidenced a novel transcriptional regulatory loop involving RIP140 and the E2F1 transcription factor. We first identify the RIP140 gene as a transcriptional target of E2F1 and demonstrate, by transient transfection of various cell lines, that overexpression of E2F1 and DP1 strongly increases transcription of the RIP140 promoter. We characterize a bona fide E2F binding site able to bind the E2F1/DP1 heterodimer in gel shift experiments and show that E2F1 is indeed recruited to the RIP140 promoter by chromatin immunoprecipitation experiments. The relevance of this regulation by E2F1 is first strengthened by the decrease in RIP140 mRNA levels in tissues from E2F1-/- mice and second, by the regulation of RIP140 mRNA expression during progression of synchronized cells into the cell cycle (peak of expression both at the G1/S and G2/M transition).

We then demonstrate that RIP140 directly interacts with E2F1 and represses its transcriptional activity on various promoters. As expected, this negative regulation of E2F1 activity is associated 1) with a significant blockade of cells in G1 upon RIP140 overexpression in MCF-7 breast cancer cells and 2) with an increased proliferation of mouse embryonic fibroblasts derived from RIP140-/- mice (as compared to wild-type cells). Very interestingly, we show that upon transformation and grafting in nude mice, RIP140-/- fibrosarcomas grow faster than controls. Finally, analysis of breast tumor samples reveals a strong inverse correlation between RIP140 expression and E2F1 target gene expression thus supporting the role of RIP140 as an important regulator of E2F1 pathway in breast cancer.

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DAX1 is a direct target of EWS/FLI1 oncoprotein and a principal regulator of cell cycle progression in Ewing tumor cells

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The molecular hallmark of the Ewing family of tumors is the presence of balanced chromosomal translocations leading to the formation of chimerical transcription factors (i.e. EWS/FLI1) that play a pivotal role in the pathogenesis of Ewing tumors by deregulating gene expression. We have recently demonstrated that DAX1 (NR0B1), an orphan nuclear receptor which was not previously implicated in cancer, is induced by the EWS/FLI1 oncoprotein and is highly expressed in Ewing tumors, suggesting that DAX1 is a biologically relevant target of EWS/FLI1-mediated oncogenesis. In this work we demonstrate that DAX1 is a direct transcriptional target of the EWS/FLI1 oncoprotein through its binding to a GGA-rich region in the DAX1 promoter and show that DAX1 is a key player of EWS/FLI1-mediated oncogenesis. DAX1 silencing using an inducible model of RNA interference induces growth arrest in the A673 Ewing cell line and severely impairs its capability to grow in semisolid medium and form tumors in immunodeficient mice. Gene expression profile analysis demonstrated that about ten percent of the genes regulated by EWS/FLI1 in Ewing cells are DAX1 targets, confirming the importance of DAX1 in Ewing oncogenesis. Functional genomic analysis, validated by quantitative RT-PCR, showed that genes implicated in cell cycle progression, such as CDK2, CDC6, MCM10 or SKP2 were similarly regulated by EWS/FLI1 and DAX1. These findings indicate that DAX1 is an important player in the pathogenesis of the Ewing family of tumors, identify new functions for DAX1 as a cell cycle progression regulator and open the possibility to new therapeutic approaches based on DAX1 function interference.

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Role of the p14ARF tumor suppressor in EGFR-mediated growth control of bronchial adenocarcinoma

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Lung cancer is the first cause of cancer death worldwide. Among non conventional treatments used in patients with bronchial adenocarcinoma are the Tyrosine Kinase Inhibitors (TKI) which inhibit activation of the Epidermal growth Factor Receptor (EGFR). The efficacy of these molecules mainly depends on the presence of mutations in EGFR (L858R, Del19). However, despite an initial response to TKIs, patients with EGFR mutations rarely achieve a complete radiographic or pathologic response and tumor cells acquire resistance to TKIs. As part of a collaborative work with the International Research Center for Cancer (CIRC) in Lyon, we recently showed that 85% of lung tumors with mutated EGFR displayed a downregulation of p14ARF expression. These observations suggest that functional inactivation of p14ARF is required in tumors with EGFR mutations and support the notion that p14ARF might protect cells against excessive mitotic signals induced by activated EGFR. On the basis of transfection experiments, we show that p14ARF can inhibit the growth of the H1975 lung tumor cell line expressing a mutant L858R EGFR. Similar results are observed in SAOS2 and H1299 cells (wild type EGFR) cotransfected with expression vectors encoding p14ARF and/or mutated L858R-EGFR. We also demonstrate that in all cases p14ARF induces the apoptosis of mutated L858R cells and show that this effect is prevented when cells are treated with ZVED, a pan-caspase inhibitor. Ras/Raf/MEK/ERK, PI3K/PDK1/Akt and JAK/STAT are the major traditionnal transduction cascades engaged by activated EGFR. We show that cell growth inhibition induced by p14ARF is prevented when cells are incubated with cucurbitacin, a specific inhibitor of STAT3 activation. By contrast, the use of Wortamin and U0126 which are specific inhibitors of the MEK/ERK MAPK and AKT pathways respectively did not alter the ability of p14ARF to restrict cell growth.

Altogether, these results show that p14ARF can protect the cells from the proliferative and antiapoptotic signals induced by mutated EGFR. Furthermore, they suggest that EGFR/STAT3 signaling pathway is be involved in the growth suppressive function of p14ARF. The molecular mechanisms involved in this process are under investigation.

1 Mounawar et al, 2007, Cancer Res., 67: 5667-72

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Ets-2 is required for hTERT gene transcription and cancer cell proliferation

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This study investigates Ets-2 mediated regulation of telomerase in cancer. Ets-2 is a member of the Ets transcription factor family, comprising over 30 members. Ets members are involved in diverse biological processes and are frequently over-expressed in cancer. Similarly, telomerase activity is exhibited in 90% of cancers, and is critical for cancer cell proliferation. Here, we show that Ets-2 is a regulator of telomerase in cancer cells by controlling the gene expression of the catalytic subunit of telomerase-hTERT (human telomerase reverse transcriptase). Using RNA interference technology, we found that silencing Ets-2 results in significant inhibition of hTERT mRNA levels and telomerase activity in multiple cancer cell lines. This coincides with increased apoptosis as determined by flow cytometry. Ectopic expression of Ets-2 or hTERT reversed both telomerase activity inhibition and cell death, induced by silencing Ets-2 which suggests inhibition of Ets-2-mediated cell death is telomerase dependent. Using chromatin immunoprecipitation assays we show that Ets-2 binds the hTERT promoter, and we have identified two non-canonical Ets binding sites in the hTERT promoter. Mutation of these sites significantly decreases hTERT promoter activity. Thus, this data supports direct activation of hTERT transcription by Ets-2. Interestingly, inhibition of Ets-2 by siRNA also decreases expression of c-Myc, a potent stimulator of hTERT gene expression. In conclusion, this data suggests that Ets-2 is a key inducer of telomerase activity. We show that Ets-2 directly and indirectly stimulates hTERT gene transcription. Therefore, Ets-2 presents an attractive target for inducing telomerase inhibition and cancer cell death.