CHAPTER 4

CtBP:
A Link between Apoptosis and the Epithelial-Mesenchymal Transition

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Abstract

Adenovirus E1a proteins are potent and ubiquitously acting tumor suppressors in human tumor cells. Through interaction with CtBP (as well as other mechanisms), E1a protein sensitizes cells to several apoptotic responses including anoikis. This interaction also induces the expression of certain epithelial cell adhesion and cytoskeletal genes in various tumor cell lines. Functionally analogous results are observed in mouse embryo fibroblasts lacking CtBP1 and CtBP2 genes. These results implicate CtBP as a potential modulator of the epithelial-to-mesenchymal transition (EMT) as well as apoptosis.

Introduction

The epithelial-to-mesenchymal transition (EMT) is an important feature of embryonic development as well as the evolution of carcinoma cells, but the relationship between EMT and the latter has, until recently, been somewhat phenomenological: cadherin/catenin signaling and cell polarity are deregulated, which somehow promotes tumor progression.[1]

More recently it has become appreciated that carcinoma cells are generally deficient in multiple apoptotic signaling pathways. In particular, their sensitivity to *anoikis*—apoptosis triggered by detachment from matrix, or attachment to the wrong matrix—is compromised (reviewed in ref. 2). The oncogenicity of EMT can now be explained because anoikis is a general feature of epithelial but usually not mesenchymal cells, so EMT programs anoikis-resistance, thus promoting tumor progression.

These combined observations frame an important question: is there a mechanistic link between the EMT and the acquisition of apoptosis-resistance? Namely, is there a specific factor or family of factors that regulates these two gene expression programs coordinately? The protein that is the subject of this book, C-terminal Binding Protein (CtBP) appears to have the properties of this factor. These properties and the indications that CtBP might represent a novel cancer drug target are summarized in this chapter.

Discovery of CtBP's Phenotypic Properties in Human Tumor Cells Using E1a as a Probe

Even though the adenovirus E1a 243 amino acid protein is oncogenic in rodent cells—primarily due to its inactivation of the retinoblastoma protein—E1a is decidedly

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CtBP Family Proteins, edited by G. Chinnadurai.

tumor-suppressive in human tumor cells due to multiple effects mediated by several protein interactions (reviewed in ref. 3).

Interestingly, E1a expression induces the expression of epithelial-specific cell adhesion and cytoskeletal genes in tumor cell lines of diverse origin, including melanoma, fibrosarcoma, rhabdomyosarcoma and others.[4] This is accompanied by the sensitization of certain cell lines to anoikis.[5,6] Mutant E1a proteins that fail to bind CtBP are partially defective in both of these effects.[5]

These results suggested that the corepressor CtBP tends to target certain epithelial-specific and apoptosis-promoting genes for repression. Indeed, our microarray analysis[7] of cells from the CtBP1,2-double-knockout mice indicated that there was such a tendency. Many other genes were regulated as well, but the only recognizable *programs* of genes that were *coordinately* induced by CtBP1,2 double knockout were epithelial-specific genes (e.g., cytokeratins and cell junction proteins), as well as pro-apoptotic genes such as PERP—which is pro-apoptotic, p53-inducible gene encoding a desmosomal protein[8,9]—and the BH3 domain protein, Noxa. CtBP is thus a corepressor for epithelial as well as pro-apoptotic gene expression, with properties of a master regulator that links these programs.

One caveat is that CtBP is known also to function as a corepressor for a very wide variety of repressor proteins, targeting lymphoid, muscle or neuronal-specific genes. This raises the question of why these genes usually are not also induced by the genetic or E1a-mediated inactivation of CtBP. A partially speculative answer is that epithelial gene promoters such as the E-cadherin promoter (which is a known target for repression by CtBP-ZEB1 and CtBP-SIP1 complexes) appear not to require tissue-specific transactivator proteins for expression, needing only ubiquitous factors such as Sp1, NF1/CTF, and others, for expression (although a novel intron 2 enhancer has recently been identified, which may potentially interact with additional activators or repressors, as yet unidentified[10]). We hypothesize that because the transactivators needed to drive their expression are ubiquitous, these promoters (in contrast with other tissue-specific promoters, e.g., muscle) are induced by the simple removal of CtBP-repressor complexes. Whether an analogous phenomenon can be generalized across the many epithelial and pro-apoptotic gene promoters that are induced when CtBP is lost remains to be seen. In the most stringent application of this model, though, "induction by loss of CtBP" may define those genes that share the simplicity of transcription factor requirements with E-cadherin.[11] Repressors for the E-cadherin promoter include Snail[12,13]and Slug[14]—which are not known to interact with CtBP (in mammalian cells)—as well as ZEB1/deltaEF1[5] and ZEB2[15] which repress transcription partly by recruiting CtBP; in some cell systems Snail induces ZEB1 expression in addition.[16] E1a is thought to de-repress the promoter by interfering with ZEB-CtBP interaction, although additional mechanisms may play a role.

Regulation of CtBP Function by Kinases and Sumoylation: Implications For Gene Expression and Apoptosis

Analogously with the control of coactivator function by kinases, the corepressor function of CtBP protein is regulated by several modifications affecting its localization, repressor interaction and degradation.

With regard to localization, CtBP has been variably reported as entirely nuclear or as partly cytoplasmic; indeed a cytoplasmic function in Golgi tubule dynamics has been proposed.[17] Thus, the localization of CtBP may prove to be somewhat cell-type-, treatment-, antibody- or detection method-dependent. Nevertheless, a recent report demonstrates that a fraction of CtBP protein is sumoylated by PIAS proteins in vitro and in vivo, and that mutation of the Sumo acceptor site (K428) virtually abolished its nuclear localization.[18] Conversely, binding of cytoplasmic PDZ-containing proteins such as nNOS to CtBP's PDZ binding domain adjacent to the Sumo acceptor site (DQL438-440) blocked Sumoylation and resulted in the cytoplasmic accumulation of CtBP. It is difficult to reconcile that only a small fraction of CtBP protein is sumoylated, while most of the CtBP is nuclear, with the simple model proposed. However, it