Pontin and Reptin, Two Related ATPases with Multiple Roles in Cancer

Otmar Huber,1 Ludovic Ménard,2,3 Valérie Haurie,2,3 Alexandra Nicou,2,3 Danièle Taras,2,3 and Jean Rosenbaum1,2

1Department of Laboratory Medicine and Pathobiochemistry, Charité-Universitätsmedizin Berlin, Berlin, Germany and 2Institut National de la Santé et de la Recherche Médicale U889; and 3Université Victor Segalen Bordeaux 2, Bordeaux, France

Abstract

Studies in model organisms or cultured human cells suggest potential implications in carcinogenesis for the AAA+ ATPases Pontin and Reptin. Both proteins are associated with several chromatin-remodeling complexes and have many functions including transcriptional regulation, DNA damage repair, and telomerase activity. They also interact with major oncogenic actors such as β-catenin and c-myc and regulate their oncogenic function. We only now begin to get insight into the role of Pontin and Reptin in human cancers.

Introduction

Pontin and Reptin are two related members of the AAA+ (ATPases associated with diverse cellular activities) superfamily sharing conserved Walker A and B motifs, arginine fingers, and sensor domains. Because both proteins were identified independently by several groups, multiple names exist in the literature and data bases (RuvB1, RvB1, Tip49a, NMP238, ECP54, TAP54α, and TH1 for Pontin; RuvB2, Tip49b, ECP51, TAP54γ, and TH2 for Reptin). Pontin and Reptin share limited homology to the bacterial helicase RuvB, which is essentially involved in the catalysis of Holliday junction branch migration. Whereas the functional importance of the ATPase activity of both proteins has been clearly established by the expression of proteins mutated in the Walker A or B motifs, there is dispute whether they are indeed endowed with helicase activity. In the recent years, a number of studies have established that both proteins are essential for the viability and development of model organisms including S. cerevisiae, D. melanogaster, X. laevis, or D. rerio.

Pontin and Reptin normally are coexpressed and frequently share common binding partners. Formation of homomeric and heteromeric interactions between Pontin and/or Reptin has been reported but heteromeric complex formation is preferred as analyzed by coimmunoprecipitation from lysates of transfected cells. In vitro, Pontin and Reptin form hexameric or double hexameric ring systems (1). Most evidence points to an association of Pontin and Reptin in the cell nucleus with several types of high molecular weight complexes involved in chromatin remodeling and/or transcriptional regulation such as, in human, the hINO80, Tip60, SRCAP, and Uhl1/Prefoldin complexes (1). It is, however, noteworthy that the multimeric status of Pontin and Reptin in the cytoplasm is not well defined. Some data even suggest that they are found in different subcellular locations in the course of cell division (2). The structural aspects of Pontin and Reptin and their functions in the regulation of transcription have been recently reviewed (1) and thus will not be addressed here in detail.

Expression in Cancer

Until recently, there were no data about the expression of Pontin and Reptin in human cancer. We performed a differential proteomic analysis of a few cases of human hepatocellular carcinoma (HCC) that were compared with the non–tumor-surrounding liver (3). This revealed an overexpression of Reptin in the index cases that led us to study a further 96 cases of human HCC with real-time PCR. It turned out that Reptin transcription was increased in 75% of the cases (same for Pontin; ref. 4) and that high levels of Reptin were correlated with a poor prognosis, independently of other prognosis variables. We have also investigated Pontin expression in a series of 34 colon cancer specimens and found it to be increased in more than 80% of the cases (5). The regulation of Reptin or Pontin expression has not been reported as such in other large series of tumors. However, reanalysis of microarray data available in the Oncomine database shows a deregulated expression of these proteins in several cancers such as bladder cancer and melanoma.

The mechanisms inducing overexpression of Reptin and Pontin in tumors are unknown and may be tumor-type specific. The Reptin gene is located on chromosome 19q13.3, a region not commonly affected in HCC, and it is thus unlikely that Reptin overexpression is consecutive to a gene amplification event. The same is true for the Pontin gene, which is located on chromosome 3q21. In contrast, Pontin is overexpressed in non–small cell lung cancer (6) where this locus is commonly amplified.

Unexpectedly, we detected strongly enhanced cytoplasmic expression of Reptin (4) and Pontin in liver cancer and of Pontin in colon cancer (5). All known functions of Pontin and Reptin are consistent with a nuclear localization, and both proteins indeed have been localized in the nucleus in many cell types from various organisms. On the other hand, both Reptin and Pontin can be found to be associated with the mitotic apparatus, although at different locations during mitosis, suggesting that they may play distinct roles during this process independently of their nuclear functions (2).

Because Pontin and Reptin expression seems to be increased in a large number of cancer types, these proteins may be of general interest for oncoologists. The specific nuclear and nonnuclear functions of both proteins remain to be dissected and may elucidate important aspects about their roles in cancer cells.

4 Unpublished data.
5 http://www.oncomine.org

Requests for reprints: Jean Rosenbaum, Institut National de la Santé et de la Recherche Médicale U889, Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux cedex, France. E-mail: jean.rosenbaum@gef.u-bordeaux2.fr.
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Interactions with Mediators of Carcinogenesis

Numerous Pontin and Reptin interaction partners have known roles in cancer. The first hints suggesting a relationship between Pontin, Reptin, and cancer came with the discovery that both proteins interacted with β-catenin (7, 8) and c-myc (9). Moreover, expression of both proteins is regulated by c-myc (9). Pontin seems to be required for the transforming effect of c-myc (9), the viral oncoprotein E1A (10), or β-catenin (11). There are no similar data available for Reptin. On the other hand, in various assays, Reptin was found to antagonize the transcriptional effect of the T-cell factor/lymphoid enhancer factor-1-β-catenin complex, whereas Pontin potentiated it (8). However, more recently it was shown in metastatic prostate cancer cells that Reptin, but not Pontin, was recruited to the promoter of the metastasis suppressor gene KAI-1 in a complex with β-catenin and was required for the repressing effect of β-catenin on the transcription of this gene, possibly contributing to the enhanced invasive properties of tumor cells (12). The formation of this complex seems to depend on sumoylation of Reptin (13). Conversely, in nonmetastatic prostate cancer cells, Pontin, but not Reptin, is recruited to the same promoter in complex with the Tip60 histone acetyltransferase, although Pontin is not required for the activating effect of Tip60 on transcription (12). On the other hand, Pontin turned out to be a cofactor for the transcriptional activation of androgen receptor target genes in a sumoylation-dependent way (14).

Tip60 is a multifaceted protein that is also involved in DNA damage repair, where it operates within a multiprotein complex containing both Pontin and Reptin (15). Their likely role in the response to DNA damage is in agreement with the recent observations that both Pontin and Reptin seem to be phosphorylated by ataxia telangiectasia mutated (ATM)/ATM and Rad3-related following DNA damage (16) and that Pontin is required for Tip60 activity after DNA damage (17).

We have also shown that Pontin and Reptin bind the tumor suppressor Hint1 (histidine triad nucleotide-binding protein 1/ protein kinase C inhibitor 1; ref. 18). Hint1 binding can disrupt the Pontin-Reptin interaction, but it is not known whether Reptin or Pontin modulates Hint1 functions. Reptin, but not Pontin, also interacts with the transcription factor activating transcription factor 2 (ATF-2; ref. 19), which can function either as a tumor susceptibility protein or as a tumor suppressor according to the cell type. Reptin interferes with the transcriptional activity of ATF-2, and this may participate in its function.

Thus, Pontin and Reptin interact with many established actors of carcinogenesis. Although they may seem to act antagonistically at the molecular level, they both are involved in events that favor tumor progression.

Roles in Cell Viability/Cell Death

As mentioned above, the absence of expression of either Pontin or Reptin impairs cell growth in several organisms. The presence of one protein cannot compensate for the deficiency of the other. Loss-of-function mutations in Walker domains have similar effects on growth as the absence of the protein, likely indicating a requirement for the ATPase activity. Conversely, overexpression of Pontin or Reptin in the Xenopus embryo leads to an increase in proliferation, an effect that is independent of the function of Walker domains but is likely instead related to interactions with c-myc (20).

Although interactions with c-myc may be involved (20, 21), the mechanisms through which Pontin and Reptin regulate cell growth remain incompletely understood. Several data point to a role in the G1 part of the cell cycle. Indeed, loss of Reptin in yeast induces a blockage in G1 (22). This is coherent with the observation that both proteins are involved in the transcriptional repression of p21, a repressor of the cyclin E/cyclin-dependent kinase-2 complex required for the G1-S transition (20). In human HCC cells, we have observed a concomitant block in G2-M after knockdown of Reptin. Intriguingly, abrogation of Pontin or Reptin expression has been shown to induce premature senescence, which may contribute to the observed effects on the cell cycle (23). This is in line with the very recent finding that Pontin and Reptin are required for the assembly and function of the telomerase complex (24).

Besides their role in regulating cell proliferation, both Pontin and Reptin are involved in the regulation of apoptosis. We observed that siRNA-mediated knockdown of Reptin in human HCC cells led to spontaneous apoptotic cell death (4). Similar results were obtained in other tumor cell lines, either hepatic or not (MCF-7). Conversely, transduction with a lentiviral vector coding Flag-tagged Reptin, which induced a moderate overexpression of Reptin, conferred an increased resistance to apoptotic cell death in HCC cells (4). Strikingly, Tytczy et al. (25) found that siRNA-mediated knockdown of Reptin in the human osteosarcoma cell line U2OS had the opposite effect in decreasing UV-induced apoptosis. This discrepancy may be explained by the fact that UV-induced cell death is dependent on p53 activation, and Reptin acts as a partner in the Tip60 complex that functions as a coactivator of p53 (25). On the other hand, we have found that the spontaneous apoptosis following knockdown of Reptin in a variety of HCC cells occurred whether p53 was wild-type or mutated (9).

Pontin also seems to act as an antiapoptotic factor. Indeed, a dominant-negative mutant of Pontin, devoid of ATPase activity, potentiates the apoptotic activity of c-myc and of E2F1 (10). We found that, similar to Reptin, the knockdown of Pontin in HCC cells led to spontaneous apoptosis. As mentioned above, Pontin and Reptin interact with Hint1, which triggers apoptosis by binding to and activating, notably, the Bax promoter (26). An attractive hypothesis is that the increased cytoplasmic levels of both proteins observed in some malignancies may sequester Hint1 in the cytoplasm, preventing it from regulating proapoptotic gene expression. Altogether, Pontin and Reptin favor cell proliferation and inhibit apoptosis, properties well in line with their supposed roles in cancer.

Pontin and Reptin as Therapeutic Targets?

As mentioned above, decreased expression of either Pontin or Reptin results in reduced tumor cell growth and increased apoptosis in vitro (4). We have also found that decreasing Reptin expression results in growth arrest of established tumors in xenograft experiments in mice. Thus, these proteins may qualify as therapeutic targets. It is also interesting to note that Reptin and

6 Unpublished data.
7 Unpublished data.
8 Unpublished data.
9 Haurie V, Ménard L, Taras D, and Rosenbaum J, manuscript in preparation.
10 Unpublished observations.
Pontin are endowed with an ATPase activity, shown to be required for most of their known functions, which could be targeted by specific inhibitors.

**Conclusion**

Pontin and Reptin remain intriguing proteins that, given their alleged functions, seem to be involved in carcinogenesis at a multitude of steps. Much remains to be understood about them, but one of the most challenging issues may be the understanding of their respective roles. Indeed, although they are most often found in the same complexes, this is not always the case (12). In addition, both proteins have been evolutionarily conserved, suggesting that they have distinctive functions, which is supported by the finding that the deficiency of one cannot be rescued by the expression of the other (9). It is likely that both the nature of the multimeric complexes they form and the partners they interact with will dictate their function in a variety of cellular processes relevant for carcinogenesis (Fig. 1).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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