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Prothymosin alpha and Tumor: Current Status and Perspective

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[ABSTRACT] Prothymosin alpha (ProTα) is a small molecule of natively unstructured acidic protein, and widely exists in mammalian tissues. Nevertheless, its biological functions are still elusive. Recent studies indicate that ProTα is involved in carcinogenesis and cancer development. We reviewed current reports on the potential roles of ProTα in cell proliferation, carcinogenesis, apoptosis, and immunomodulatory, discussed the regulation of ProTα gene expression and possible molecular mechanisms underlying its internal and external actions in cells, and explored its significance in tumor diagnosis and treatment.

KEYWORDS: Prothymosin alpha; Neoplasm; Tumorigenesis; Apoptosis; Transcription factor; Diagnosis; Treatment

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Received: 2006-06-21
Revised: 2006-08-14

Prothymosin alpha (ProTα) was first isolated from rat thymus in 1984, and was originally regarded as a thymic hormone. Then it is found virtually exclusively in the nuclei of all mammalian cells and tissues. Human ProTα is a 12.4 ku polypeptide, containing 109 amino acids, with a large proportion of acidic amino acids. The peculiar amino acid composition of ProTα results in its exceptional acidity (pI 3.5) and hydrophilicity to form an unusually unfolded, natively unstructured protein, which is negatively charged with poor immunogenicity. A nuclear localization signal (NLS) is located close to the carboxyl terminus of the ProTα molecule. ProTα is evolutionarily conserved and is currently the only protein in mammals reported to bear phosphoglutamate. cDNA Library reveals that human ProTα is one of the plentifully expressed genes in cells. Its high conservation in mammals and broad tissue distribution suggest that ProTα serves essential biological functions. However, until now, the exact physiologic functions of ProTα are not fully elucidated. The only one definite function is its relationship with the proliferation of the cells and the requirement of it for the cellular growth and survival. ProTα deficient cells can not divide. In recent years, accumulating evidence indicates that ProTα is involved in tumorigenesis, apoptotic activity and transcriptional regulation. Nowadays, the research is to investigate the role and mechanisms of ProTα in cancer diagnosis and treatment.

The relation of ProTα and tumors as well as possible mechanisms

ProTα works as an oncogenic protein

Orre et al. [1] showed that heterologous expression of ProTα in rodent fibroblasts is sufficient to induce a typical transformed phenotype of these cells *in vitro,* which is characterized by

increased proliferation, anchorage-independent growth, disappearance of contact inhibition, and decreased serum dependence of the transfected Rat-1 cells. The results suggest that ProTα may function as a cellular oncogene and is likely to be one of the important downstream targets for transformation inducers. Additionally, it was reported that ProTα expression levels were increased with progression from normal epithelium, through prostatic intraepithelial neoplasia to carcinomas, suggesting that ProTα expression is intimately involved in the differentiation and progression of human prostate cancers [2]. Although the exact function mode of ProTα action remains unclear, recent work has shown that ProTα is involved in transcriptional regulation [3, 4]. It has shown that ProTα is involved in transcriptional regulation at the level of histone acetylation through its interaction with transacetylase p300 and Epstein-Barr virus (EBV) nuclear antigen (EBNA) 3C [5,6].

ProTα works as a programmed cell death (PCD)-associated protein

It is recently found that a high level of ProTα production appeared in cancer cells may be beneficial both for maintaining a high proliferation rate and for protecting these cells from apoptosis. The *in vivo* and *in vitro* experiments documented that in apoptotic HeLa cells, ProTα is cleaved by caspase-3 at its carboxy (C)-terminus (at D99) to generate truncated ProTα (tProTα), which lacks the nuclear address, so that tProTα accumulates in cytosol [7], leading to a profound alteration in subcellular localization of the truncated protein, to weaken its intranuclear proliferation-related function. This phenomenon occurs in the apoptotic cells, but not in normal cells. Moreover, recent reports also demonstrate that ectopic production of ProTα and its derivatives with nuclear or nuclear-cytoplasmic localization protect HeLa cells from apoptosis, induced by the tumor necrosis factor (TNF) [8]. This antiapoptotic nature of ProTα is consistent with the conclusions drawn by Jiang et al [9], that ProTα negatively regulates caspase-9 activation by inhibiting apoptosome formation. Additionally, ProTα is shown to be involved in the protection of cells against apoptosis and in the regulation of expression of the oxidative stress-protective genes [10]. Moreover, ProTα may promote cell survival through Bcl-2 anti-apoptotic pathway [11]. More recent studies have found that two natively unstructured proteins with anti-apoptotic activity, p8 and ProTα, are in fact borne by the p8/ProTα complex, which are individually inactive [12]. These results show that the natively unfolded proteins may exert their functions through a multi-protein complex form.

Different results about the roles of ProTα in apoptosis have been reported, which suggest a possible role of tProTα in the development of reactive oxygen species (ROS)- and cytochrome c-mediated apoptosis. During apoptosis, negatively charged tProTα is cleaved by caspase-3 and interacts with positively charged cytochrome c in cytosol to form a complex of cytochrome c/tProTα. The complex formation is found to abrogate all the antioxidant functions of cytochrome c. Furthermore, cytochrome c complexes can operate as pro-oxidants because they become autoxidizable. Such events might be favorable for the development of apoptosis. However, it seems that the effect of tProTα depends upon its level in cytosol. When the concentration of tProTα is below a certain level, it might increase (ROS) by the above described mechanisms; when the concentration is above a level, tProTα might compete for apoptotic protease activating factor-1 (Apaf-1) with cytochrome c to inhibit apoptosis[13].

Type-1 cell death (apoptosis) and type-2 cell death (autophagy) are two most prominent morphological and biochemical subtypes in both physiological and pathological status. There is a correlation between underactivity of autophagy and the development of certain kinds of tumors. Autophagy and apoptosis may oppose or promote each other under certain conditions, and also can take place consequently or coexist in a cell at the same time. It is possible that mitochondrial dysfunction elicited by an apoptotic stimulus can activate autophagy when caspase activation is inhibited. Recently, it is proposed that ProTα may have additional pathogenic effects related to the switch between apoptosis and autophagy. Apoptosis or autophagy program depends on the cell environment. ProTα might act as a switch between the two kinds of PCD by inhibiting the assembly of the apoptosome and the caspase activation, and high levels of ProTα might induce autophagy,

suggesting that autophagy revoked by ProTα would bring both positive and negative effects on tumor cells.

Regulation of the expression of ProTa gene

The signal pathway controlling ProTα expression in cells is still unknown. To date, it is indicated that transcription factor c-myc and E2F are strong positive regulators of ProTα gene; tumor-inhibiting factor p53 works as a negative regulator for ProTα regulation. ProTα has also been proposed to be a target of the *c*-myc protooncogene via an E-box element (CACGTG) localized in the first intron of the gene. *c*-myc is a proto-oncogene that has been implicated in diverse forms of tumorigenesis. It has been reported that overexpression of ProTα concomitant with c-myc are found mainly in hepatoma and colorectal cancers. Growth promotion protein E2F can directly control the expression of ProTα in cell cycle by activating a reporter gene of ProTα promoter. ProTα is accommodated negatively by tumor-inhibiting factor p53 [15]. Recent reports further indicate that ProTα elicits a p53 response that is characterized by the increased p53 transcriptional activity and acetylation of p53. And overexpression of ProTα leads to increased mRNA and protein levels of endogenous p53 [16].

Recently, it is demonstrated that the ProTα gene is a primary responser gene to estrogen. ProTα is positively regulated by estradiol (E_2) in estrogen receptor (ER)-positive breast cancer and neuroblastoma cells at both mRNA and protein levels [17,18], which underscore the importance of ProTα in estrogen-induced breast cell proliferation. E_2 treatment induces the transcriptional activity of estrogen-responsive elements in ProTα promoter, and activates the gene transcription [19,20]. ProTα is also found to promote the transcriptional activity of ER, especially to increase the magnitude of ER alpha transcriptional activity. However ProTα does not directly interact with ER, but works as an activity-modulating protein of the estrogen receptor (ER) complex[21].

Immunostimulatory and anticancer activities of ProTα

Although ProTα has been proved to be a nucleic protein, there still exists the possibility that it has an extracellular localization. A number of functional studies suggest that this peptide exhibits immunostimulatory and anticancer activities. ProTα is considered to originate from the ruptured dead cells. And the ProTα specific receptor has been identified in the membrane of PHA-activated lymphoblasts [22]. An *in vitro* study found that when various concentrations of ProTα were exogenously administered in the culture of splenocytes, ProTα increased the secretion of interferon (IFN)-gamma, IFN-alpha and TNF-alpha [23]. IFNs play critical roles in host defense by modulating the expression of various genes via tyrosine phosphorylation of STAT transcription factors, which regulates the gene expression in the acute phase. It is identified that ProTα interacts with the amino terminal of tyrosine-phosphorylated STAT3 [24]. A number of functional studies suggest that a set of N-terminal ProTα peptides, such as thymosin α 1 (ProTα residues 128) and thymosin α 11 (residues 135), have extracellular localization and immunostimulatory activities *in vivo* and *in vitro*. Recently, Skopeliti et al. [25] reported that they identified the peptide segment of ProTα presenting maximal immunomodulatory activity at its several carboxy (C)-terminus, generating potentially bioactive fragments. The fragments exhibited the ability to stimulate the proliferation of healthy donor- and cancer patient-derived peripheral blood mononuclear cells (PBMC) in autologous mixed lymphocyte reaction (AMLR), enhance the activity of natural killer and lymphokine-activated killer cells, increase the intracellular production of perforin, upregulate adhesion molecules and CD25 expression. The mechanisms of intranuclear and extrocellular actions of ProTα may be distinct.

Molecular mimicry hypothesis-a possible mechanism of ProTα function

ProTα is a natively unstructured protein, appearing to possess a rare random coil conformation, and, lacking of the secondary structure in solution. But exogenous ProTα is always used in solution in *in vitro* and in vivo experiments. Pineiro et al. [26] proposed a molecular mimicry hypothesis, suggesting that the molecular mimetism may be the underlying basis. Notwithstanding it is clear that the function of a protein is depending on its tertiary structure. Some proteins form their tertiary structures merely when they interact with other molecules. In addition, there are

two ways to validate the molecular mimicry hypothesis: one is using the long acidic amino acid sequence, accompanied by the change of the electrostatic interaction; and another is through its homology to other non-associated proteins at both C- and N-terminus. Moreover, we must consider the possibility that ProTα adopts the specific structure under specific cellular conditions. If ProTα changes in conformation due to the environment, it may start to secret, which would help to explain that an electronegative protein could cross the plasma membrane despite of the absence of a secretion signal. On the other hand, folding in the membrane might affect ProTα -receptor interaction. Moreover, it cannot be excluded that this interaction itself might induce some kinds of folding, and thus confer functions to the molecule. In summary, the molecular mimicry hypothesis provides a clue that can not be overlooked for a realistic appraisal of the results, which are related to the functions of the natively unstructured proteins.

ProTα acts as a tumor marker

It has previously been shown that ProTα is overexpressed in some neoplasms, and the expression level is associated with prognosis, metastatic potential, and the overall survival of the tumor. In the breast cancer tissues, ProTα concentration is 17-fold higher than that in normal breast tissues, and ProTα has been shown as a prognostic value as it is associated with the metastatic potential of the tumor and also with the risk of the death [27]. Likewise, ProTα serves as a prognostic marker for hepatocarcinoma [28], lung cancer [29] and neuroblastoma [30]. ProTα is found to be a potential biomarker for colon cancer after analyzing 39 cancer cell lines from 9 different tissues using ProteinChip technology [31]. Recently, a study showed that ProTα expression was significantly elevated in well-differentiated carcinomas compared to adenomas, goiters and normal tissues [32]. More recent data reveal the role of ProTα as a specific tumor marker for the detection and following up of transitional cell carcinoma of the urinary bladder before and after the treatment. Tzai et al. [33] quantified the ProTα level in urine by enzyme-linked immunosorbent assay (ELISA) and found that the urine ProTα level in newly diagnosed bladder cancer patients without any treatments was significantly higher than that in those who were tumor free after the treatment. And the urine ProTα level was constantly elevated in the residual or recurrent tumors after the treatment. Moreover, ProTα expression is related to the intratumoral vascularization and the aggressive behavior of the pituitary tumor [34]. ProTα is also possibly involved in the adhesion, migration and proliferation of human ovarian cancer [35].

The perspective application of ProTα in diagnosis and therapy

Because there is a close correlation of ProTα level to the proliferation of tumor cells, ProTα may have an extensive application perspective in tumor diagnosis and treatment. Because of the peculiar amino acid composition and poor immunogenicity of the protein, ProTα concentration in tissues was measured by radioimmunoassay (RIA) and ELISA methods. A recent study have shown that a commercially designed, highly specific anti-prothymosin monoclonal antibody against human ProTα molecule was obtained and characterized [36], which is suitable for the immunolocalization of the endogenous ProTα within the cells.

ProTα gene may become one of the possible targets for pharmacogenomics in the field of oncology. Specifically, alpha-(trichloromethyl) -4-pyridineethanol (PETCM) screened as an activator of caspase [9] settled the foundation for developing the original antineoplastic agents. A previous study demonstrated that soy isoflavones is capable of inhibiting prostate carcinogenesis of rats by the down-regulation of ProTα [37]. ProTα is also identified as a novel potential target of ursodeoxycholic acid (UDCA) by GeneChip microarray [38]. The prospect of inducing apoptosis selectively in cancer cells is obviously attractive and is presumably the rationale for ProTα -specific gene therapy. It is demonstrated that the knockdown of ProTα expression by antisense oligonucleotides or RNA interference (RNAi) could inhibit cell proliferation in myeloma and PC3 cells *in vitro* [39,2] and induce apoptosis in HL-60 cells [40]. On the other hand, as an immunopotentiator, the application of ProTα *in vitro* has achieved noteworthy efficacy. Judging from these results, clinical trial of ProTα on cancer patients is the next step in this field. In addition, both the commercial production and recombinant expression will provide large quantities of ProTα protein as required for this application [41].

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