World Journal of Experimental Medicine

Online Submissions: http://www.wjgnet.com/2220-315Xoffice
wjem@wjgnet.com
doi:10.5493/wjem.v1.i1.7

World J Exp Med 2011 December 20; 1(1): 7-9
ISSN 2220-315X (online)

OBSERVATION

Neuroendocrine differentiation and the ubiquitin-proteasome system in cancer: Partners or enemies?

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Received: September 13, 2011 Revised: December 9, 2011
Accepted: December 16, 2011
Published online: December 20, 2011

Abstract

Neuroendocrine (NE) differentiation of cancer and deregulation of the ubiquitin-proteasome system (UPS) are two processes that have been independently linked to the development of aggressive and treatment-resistant tumors. Striking data suggest a plausible interconnection between these two mechanisms, based on indirect evidence of neuropeptide-induced effects on UPS, reversed by proteasome inhibition and deubiquitinase-like properties of NE markers. Deciphering the model of their exact interactions is one of the keys to targeting the NE malignant phenotype more effectively.

Key words: Neuroendocrine differentiation; Ubiquitin; Proteasome; Cancer

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Vlachostergios PJ, Papandreou CN. Neuroendocrine differentiation and the ubiquitin-proteasome system in cancer: Partners or enemies? *World J Exp Med* 2011; 1(1): 7-9 Available from: URL: http://www.wjgnet.com/2220-315X/full/v1/i1/7.htm DOI: http://dx.doi.org/10.5493/wjem.v1.i1.7

The study of neuroendocrine (NE) cancers and NE differentiation of solid tumors has been hampered by a number of circumstances, including our limited understanding of the cellular and molecular biology of NE cells and the mechanisms of tumorigenesis, a shortage of *in vitro* and animal models to study disease pathogenesis and treatment, a paucity of critical targets for new therapies, and a lack of uniform pathological classification and staging systems. The carcinoid tumor group is heterogeneous and comprises a mixture of foregut-, midgut- and hindgut-derived tumors. Furthermore, clinical studies are commonly performed on patients with tumors displaying varying degrees of differentiation, proliferation rates and disease stage, thus limiting the validity of the conclusions drawn. Experimental data from well-characterized *in vitro* and animal models are therefore needed to properly evaluate novel treatment principles[1].

The ubiquitin-proteasome system (UPS) constitutes a large multiprotein complex present in all cells, which degrades damaged, oxidized or misfolded proteins which are targeted for ubiquitination. Furthermore, it orchestrates the orderly degradation of regulatory proteins that govern cell cycle, transcription factor activation, apoptosis and cell trafficking[2]. A classic example is regulation of nuclear factor κB (NFκB), a key transcription factor promoting cell survival, angiogenesis and metastasis, related to cancer progression and resistance to chemotherapy in various solid tumors. NFκB activation is dependent on proteasome-mediated degradation of the NFκB inhibitory protein IκB[3].

Coincident with progression from prostate cancer *in situ* to metastatic disease is an increase in the number of tumor cells exhibiting NE differentiation. NE cells express a variety of hormone peptides, including endo-

thelin-1 (ET-1), the bombesin (BBS)-like peptide, gastrin-releasing peptide, and their receptors[4]. Although there is a strong positive correlation between the degree of NE differentiation and the metastatic potential of prostate cancers, a mechanistic link between increased expression of markers of NE differentiation such as neuropeptides and the UPS had not been established before the study of Levine *et al*[4] who demonstrated that BBS treatment induced IκB degradation, NFκB translocation to the cell nucleus, increased NFκB DNA binding and upregulation of expression of pro-angiogenic factors IL-8 and VEGF, whereas proteasome inhibition by MG132 blocked these effects. These were the first data suggesting a positive modulatory role of neuropeptides in UPS regulation. Later preclinical studies reported a remarkable activity of the proteasome inhibitor bortezomib against several NE tumor cell lines of pancreatic and bronchial origin, with IC_{50} values of the drug < 1 μmol/L[5]. The mechanism of action of bortezomib in these cell lines was supported to be induction of apoptosis *via* activation of the extrinsic apoptotic pathway at longer exposure times (> 24 h), while at shorter drug exposure times (24 h) the intrinsic apoptotic pathway, resulting in nuclear condensation and fragmentation[6].

Treatment of human pulmonary and gastrointestinal carcinoid cells with MG132 resulted in growth inhibition and apoptosis which was in parallel with a dose dependent inhibition of NE markers chromogranin A and Achaete-Scute complex-like 1. These effects also coincided with an increase in the level of phosphorylated Glycogen Synthase Kinase-3β (GSK-3β), which is a highly active serine/threonine protein kinase in carcinoid cells. Phosphorylation of GSK-3β has been shown to inhibit NE tumor growth and the carcinoid phenotype[7].

Furthermore, expression of the neuron cytoplasmic protein gene product 9.5 (PGP9.5)/ubiquitin C-terminal hydrolase 1 (UCHL-1) was found elevated in multiple myeloma cells and primary specimens and was suggested as a potentially useful marker for the screening of proteasome inhibitor sensitivity, given its involvement in deubiquination as a thiol protease that recognizes and hydrolyzes a peptide bond at the C-terminal of ubiquitin[8]. However, PGP9.5 is also known as a specific tissue marker for the NE system and here lie implications for possible associations between NE phenotype and UPS. It was the same protein that was found consistently upregulated both at the gene and protein level in airway epithelial samples of smokers compared with non-smokers. UCHL1 expression was evident only in NE cells of the airway epithelium in non-smokers but was also expressed in ciliated epithelial cells in smokers. It was therefore suggested that the overexpression of UCHL1 in chronic smokers, combined with previous data of UCHL1 overexpression in > 50% of lung cancers, may represent an early event during lung carcinogenesis[9]. The already established role of UCHL1 in the degradation of unwanted, misfolded or damaged proteins within the cell might imply that an increased deubiquitination hallmarks NE differentiation and transition to a NE phenotype. This scenario may further be enriched by our recent *in vitro* results in androgen-independent prostate cancer, consistent with an increased proteasomal activity, pronounced NFκB activation and increased levels of secreted ET-1. The exact mirror image was observed in androgen-dependent prostate cancer cells displaying low secreted ET-1 levels and a low-level activation of the NFκB pathway associated with low 20S proteasome activity[10]. Thus, it seems that during transition to a NE differentiated, androgen-independent state, neuropeptide abundance coincides with upregulation of proteasome activity.

The induction of both ubiquitination-mediated protein degradation and deubiquitination processes in the context of a NE phenotype may seem difficult to explain at first. However, numerous proteins with divergent roles within the cell are targeted by the proteasome, including proteins related with NE differentiation. In this case, an elevated deubiquitinase activity would be needed to protect such proteins from being degraded and thus maintain a NE phenotype. BCCIP is a proteasome-substrate protein, whose overexpression in prostate tumor cells induces an apparent NE differentiation. BCCIP degradation is promoted by overexpression of its associated protein, LYRIC/AEG-1, and blocked by proteasome inhibition[11].

It is therefore evident that preclinical data support a bidirectional association between UPS and NE phenotype, possibly enabling us to consider them as partners, although this remains to be further elucidated. In the era of targeted anticancer therapies, this association provides a rational for testing the use of proteasome inhibitors in these tumor types at the clinical level. A first attempt was made in a small phase 2 study of bortezomib at a dose of 1.5 mg/m^2 on days 1, 4, 8 and 11 every 21 d in patients with metastatic NE (carcinoid and islet cell) tumors. As a result, disease stabilization was seen, although no partial or complete remissions were observed. This might at least partially be due to an inadequate dosing schedule as the mean percentage of 20S proteasome inhibition achieved in whole blood at 1 and 24 h after bortezomib administration was 68% and 30%, respectively, thus reducing therapeutic efficacy[12].

More importantly, an in-depth analysis of the molecular part of NE differentiation which involves UPS components (ubiquitin ligases, ubiquitin-like modifiers, deubiquitinating enzymes, proteasome subunits) and how these components interact with neuroendocine proteins modulating their stability, is anticipated to offer a better understanding of the UPS - NE phenotype association, thus resulting in better clinically-oriented research. Hopefully, research directed to identifying the particular subpopulation of patients with a particular cancer prior to intervention would allow for a selective process and potentially a more favorable clinical outcome.

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S- Editor Wang JL **L- Editor** Roemmele A **E- Editor** Zheng XM