

Phospho-TCTP and Dihydroartemisinin: A Novel Therapeutic Opportunity in Advance Breast Cancer

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Abstract

The development of resistance to chemotherapeutics is still a severe event in cancer patients. One of the major obstacles to the effectiveness of cancer therapies is the development of side effects and resistance to therapy. The characterization of novel biomarkers that support a more aggressive phenotype may provide new diagnostic tools and new opportunities for cancer therapy. In the context of breast cancer disease, the phosphorylated form of Translationally controlled tumor protein (phospho-TCTP) appears a new prognostic factor for aggressive breast cancer and tumor progression. Dihydroartemisinin (DHA) is a novel approach to molecular targeted therapies, or combination regimens in advance breast cancer.

Keywords: Advanced breast cancer; Phospho-TCTP; PLK1; DHA; Target therapy

Commentary

Translationally controlled tumor protein (TCTP) is a highly conserved protein [1], and it has been implicated in different physiological processes including apoptosis, cell proliferation and resistance to stress responses [2,3]. Despite numerous reports suggesting relevant functions of TCTP in the context of tumor biology, and many findings highlighting that TCTP overexpression is associated with tumor progression and poor clinical outcome in many poorly differentiated tumors [4-7], the precise role of this protein is still a matter of debate. An important observation, which will be helpful to elucidate its implication in cell survival mechanisms, is that TCTP is a direct substrate of the serine/threonine kinase polo-like kinase 1 (PLK1), a crucial player in cell-cycle regulation during mitosis [8]. The precise role of phospho-TCTP during mitosis is still unclear, but it has been suggested by Yarm that TCTP-phosphorylation is a critical event for the dynamic of spindle microtubules and for a proper anaphase progression (exit from mitosis) [9].

The clinical relevance of phospho-TCTP and PLK1 expression was suggested by a recent finding showing high levels of both proteins in neuroblastoma from patients with adverse prognostic factors and poor prognosis [10]. Overexpression of PLK1 was also reported in a variety of cancers [11], including breast cancer, in which PLK1 overexpression is correlated with TP53 mutation and poor clinical outcome in primary breast cancer [12]. Notably, it has been pointed out by Wierer et al. that basal levels of PLK1 are needed to maintain a tumor suppressive transcriptional program in estrogen-dependent breast cancer cells [13]. Conversely, Bhola et al. have shown that PLK1 has lost this negative function in hormone-independent ER+ breast cancer cells, and it is a crucial kinase whose overexpression contributes to hormone-independent transcriptional activity and tumor cell viability. In addition, in tumor biopsies from patients resistant to estrogen deprivation, PLK1 protein was correlated with high Ki-67 levels (a

marker of cell proliferation), thus highlighting its role in highly proliferating and aggressive breast cancer cells [14]. As it has been indicated that only a direct inhibition of PLK1 activity impairs TCTP phosphorylation [15], the definition of phospho-TCTP activity in the context of breast tumor biology may be critical to set up new molecular targeted therapies or combination regimens for advanced and aggressive cancer. Our experience suggests the potential use of phospho-TCTP as a new prognostic factor in the clinical management of patient cohorts with a more aggressive breast cancer disease. The first interesting observation from our study is that in mammary carcinoma cells TCTP is phosphorylated by PLK1, and inhibition of PLK1 activity leads to the inhibition of cell proliferation and the reduction of phospho-TCTP levels. Moreover, in 85 human breast cancer specimens we found a clear difference in the nuclear phospho-TCTP expression between low- and high-grade breast tumors, in agreement with the data observed in neuroblastoma, suggesting a possible application of phospho-TCTP as new marker in several tumor types. The high phospho-TCTP status was also correlated with worse pathological parameters, including the Ki-67, highlighting its implication on cell proliferation of malignant cells with aggressive behaviour. Notably, both in neuroblastoma and in breast specimens, only few cells into the whole tumor compartment were positively stained with phospho-TCTP. In addition, we found that this percentage of cells increased in HER2 overexpressing tumors with a poor clinical response to trastuzumab therapy. Taken together, these findings suggest that the protein phospho-TCTP may be a marker of tumor cells with a growth/survival advantage and a drug-resistant phenotype, therefore, targeting these cells may improve long-term clinical outcomes.

In our experience, the inhibition of phospho-TCTP activity by Dihydroartemisinin (DHA) enhanced the efficacy of conventional chemotherapeutic drugs in human breast cancer cell lines with more aggressive phenotypes (i.e. HER2 overexpressing cells or with a basal like phenotype), suggesting a rationale for the design of similar combination protocols for clinical studies.

DHA is the active metabolite of Artemisinin, the active principle in *Artemisia annua*. Artemisinin and its derivatives are a family of sesquiterpene trioxane lactone, which have been extensively studied as anti-malarial agents with a good tolerability and safely profile. On October 5, 2015, Dr Youyou Tu was awarded the 2015 Nobel Prize in Physiology or Medicine for her discovery of Artemisinin as a new effective therapy for malaria. Beyond the "anti-malarial effect", these pharmacological compounds show antitumor properties as well [16]. Despite the molecular mechanisms underlying their cytotoxic effect is not clearly elucidated, these compounds are selectively cytotoxic to cancer cells, as shown by both *in vitro* and *in vivo* evidence [17]. One mechanism that has been suggested is that the highly reactive endoperoxide moiety of Artemisinin is activated by an iron source (in the form of Fe²⁺ or heme, or both) to produce reactive oxygen species (ROS) [18]. Molecular and epidemiological evidence show that iron metabolism is altered in many human cancers, including breast, colorectal, prostate, lung cancer and hepatocellular carcinoma [19], thereby making them more vulnerable to the cytotoxic effect of DHA compared to normal tissue. Moreover, promising results obtained from *in vivo* studies (xenograft tumors) in various tumors, such as colorectal or hepatocellular carcinoma [17], have provided the rationale for the design of clinical studies. Today, phase I clinical trials of Artesunate (ARS), are conducted in patients with hepatocellular carcinoma (<https://clinicaltrials.gov/ct2/show/NCT02304289>), and with colorectal cancer (CRC) (<http://www.controlled-trials.com/ISRCTN05203252>). Interesting, a randomized, double blind study to test the anti-properties of oral ARS in CRC shows a reduction in Ki-67 staining of tumor cells after ARS treatment. During a median follow-up of 42 months, one patient in the artesunate and six patients in the placebo group developed recurrent CRC [20]. In addition, a pharmacokinetic study of ARS and DHA has been performed in patients with metastatic breast cancer [21]. All together these studies may provide crucial information not only on the anticancer effect and tolerability of artesunate and DHA, its active metabolite, but also on the pharmacokinetics parameters during long-term daily administration.

Interesting, a dysregulation of iron metabolism is also found in Glioblastoma cells (GBM). GBM is a devastating malignant primary brain tumor, characterized by high resistance to conventional cytotoxic chemotherapy and radiotherapy, and despite various efforts many therapeutic approaches remain palliative. The expression of both transferrin receptor protein 1 (TfR1) and transferrin receptor protein 2 (TfR2) is upregulated in GBM and induces a dysregulation of iron metabolism, which in turn promotes the generation of reactive oxygen species (ROS) [22]. In this context of iron dysregulation, DHA-toxicity may be enhanced, and may cause a severe oxidative stress leading to apoptosis in aggressive glioma cells. The potential therapeutic application of DHA for GBM is suggested by many findings showing that: i) DHA potentiates the cytotoxic effects of Temozolomide, a chemotherapy drug commonly used in treating patients with malignant brain tumors [17]; ii) DHA reduces Hypoxia-inducible factor 1 alpha (HIF-1 α) expression and its target gene protein, vascular endothelial growth factor (VEGF) [17]; iii) DHA enhances radiosensitivity of human glioma cells *in vitro* [23]. Notably, high TCTP expression in glioma was significantly associated with advanced pathological grade, and Kaplan-Meier analysis showed that patients with glioma and higher TCTP expression tend to have shorter overall survival time [5]. More focused studies should be performed in order to establish whether DHA exerts a selective cytotoxicity by targeting a specific therapy-resistant cell compartment, and to establish whether

its cytotoxicity may induce a clinical response in patients with aggressive GBM.

Whereas the DHA cytotoxicity is correlated with a specific molecular target or by a more general mechanism through the generation of ROS is still not well defined, and further studies are needed to clarify the molecular mechanism of DHA, our study suggests that the reduction of phospho-TCTP levels by DHA, in aggressive breast cancer cells harbouring mutated p53, is a critical event that induces apoptosis, thus providing a novel approach for molecular targeted therapies, or combination regimens. Understanding the mechanisms on the antitumor effect of DHA in GBM may open new therapeutic opportunities for this threatening disease and may help in developing a new approach to overcome resistance.

Conclusion

All these data suggest that phospho-TCTP is a new promising prognostic factor for high-proliferating and aggressive breast cancer, therefore, targeting these cells may improve long-term clinical outcomes. Further studies are needed to validate phospho-TCTP as prognostic factor, and to define the dose regimens, long-term use and possible effects of DHA alone or in combination regimens. Nevertheless, DHA is a cheap, safe, and orally bioavailable drug, paving the way for exploring new therapeutic opportunities for advance breast cancer.

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