

Commentary

## Biomarker-based Diagnosis and Personalized Medicine for Cancer Patients: Trinity of Nucleolin Guided, Endostatin Treatment, and Plasma Hsp90 $\alpha$ Monitored

Wei Liu<sup>1,2,3</sup>, Yan Fu<sup>1,2,3</sup> and Yongzhang Luo<sup>1,2,3\*</sup>

<sup>1</sup>The National Engineering Laboratory for Anti-Tumor Protein Therapeutics, Tsinghua University, Beijing, China

<sup>2</sup>Beijing Key Laboratory for Protein Therapeutics, Tsinghua University, Beijing, China

<sup>3</sup>Cancer Biology Laboratory, School of Life Sciences, Tsinghua University, Beijing, China

\*Corresponding author: Yongzhang Luo, School of Life Sciences, Tsinghua University, Beijing 100084, China, E-mail: yluo@tsinghua.edu.cn

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## Commentary

Cancer is a major public health problem across the society, and is the second leading cause of death globally [1]. Early diagnosis of cancer effectively decreases the mortality associated with cancer metastasis [2]. However, the scarcity of more sensitive biomarker has hampered this important public healthy strategy. In this communication, we will discuss: 1) the topic on plasma Hsp90 $\alpha$  as a novel cancer diagnosis biomarker; 2) the present knowledge regarding the underlying regulatory mechanism of Hsp90 $\alpha$  translocation to cell membrane and secretion; 3) our strategy for cancer prevention with an emphasis on trinity of nucleolin guided, endostatin treatment, and plasma Hsp90 $\alpha$  monitored.

The intracellular heat shock protein 90 (Hsp90) is well-known and widely studied as the essential and ubiquitously expressed molecular chaperone [3-6]. It accounts for 1-2% of cellular proteins in normal cells or 2-7% in tumor cells [7]. In concert with co-chaperones and accessory proteins, Hsp90 mediates remarkably versatile activities including intracellular signal transduction [8,9], protein folding [10], cell apoptosis [11], chaperone mediated autophagy [12], antigen presentation [13], and morphological evolution [14,15]. Until now, more than 300 Hsp90's diverse "clients" have been reported to be involved in these processes in both normal cells and cancer cells [16] (for a comprehensive review of Hsp90's clients, see http://www.picard.ch/downloads/Hsp90facts.pdf and the database of the Hsp90Int, see http://www.picard.ch/Hsp90Int).

However, the discovery of secreted Hsp90 is a relatively recent story. The widely accepted specific isoform which can be secreted is heat shock protein 90alpha (Hsp90a) [17-29], whereas some researchers have also identified that heat shock protein 90beta (Hsp90 $\beta$ ) localize outside of certain cell types [20,30,31]. Work by Jay and colleagues has provided the most compelling evidence that Hsp90 $\alpha$ , not Hsp90 $\beta$ , can be detected extracellularly with functional proteomic and specific anti-Hsp90 $\alpha$  antibody [24]. After that, two pools of secreted Hsp90 $\alpha$ , including "cell surface-bound" Hsp90 $\alpha$  and "extracellular" Hsp90 $\alpha$  (eHsp90 $\alpha$ ), have been characterized from cancer cells [7]. The function of eHsp90 $\alpha$  in regulating tumor invasion and metastasis will not be covered in this short communication, which has been well summarized in recent reviews [7,32].

Previous work from our group has firstly reported the underlying regulatory mechanism of Hsp90 $\alpha$  secretion [27]. Residue Thr-90 phosphorylated by protein kinase A will disrupt the interaction between Hsp90 $\alpha$  and proteins containing tetratricopeptide repeat domains, which leads to the exposure and cleavage of C-terminal

EEVD motif. This process will initiate the downstream secretion of Hsp90α. PLCγ1-PKCγ signaling axis mediates Hsp90α plasma membrane translocation [28]. Hsp90α has no to-be-secreted signal peptide, and the presently well accepted secretory pathway is through exosome [21,33,34]. Our group reported that overexpression of PKCγ [28] or Rab3D [35] can regulate the secretion of Hsp90α through the increase of exosome release. Furthermore, the level of plasma Hsp90α is positively correlated with tumor malignancy in cancer patients [27].

Lyden's group reported that VEGFR1-positive hematopoietic bone marrow progenitors initiated the pre-metastatic niche to facilitate tumor metastasis [36]. We also observed that, at the pre-metastatic stage, the permeability of pulmonary vasculatures and extravasation of circulating tumor cells were increased. Then we used microarrays to systematically examine the gene expression in lung mesenchyme, and found that angiopoientin 2 (Angpt2), matrix metalloproteinase 3 (MMP3), and MMP10 were upregulated. These three genes showed a synergistic effect on disrupting vascular integrity in both *in vitro* and *in vivo* models [37]. In addition, we also found that local miR-30 family were deregulated in the pre-metastatic lung to promote the hyperpermeability of the lung vascular by targeting Skp2 [38]. All these studies indicate that metastasis at molecular level occurred before tumor metastasis, which provides the evidence supporting the detection of early-stage cancer.

These findings promote us to test whether detecting cancers at early stage using plasma Hsp90 $\alpha$  is feasible. Therefore, our lab and collaborators developed a quantitative detection kit for plasma Hsp90 $\alpha$  based on ELISA, and clinical trials with the enrollment of 2,347 cases demonstrated that plasma Hsp90 $\alpha$  is a novel lung cancer biomarker [39]. Besides, our small-scale clinical investigation also revealed that the levels of plasma Hsp90 $\alpha$  are all elevated in detected 16 common cancer types (unpublished data).

Liver cancer, mainly consisting of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC), has become the sixth most common malignant disease and the second leading cause of cancer-associated death [40]. Recently, we extended the ELISA kit for the auxiliary diagnosis and efficacy monitoring of patients with liver cancer patients, and an official (registered at ClinicalTrial.gov: NCT02324127), large-scale (1,647 enrollments), and multicenter (three independent hospitals) clinical trial has been accomplished. At the optimum diagnostic cutoff 62 ng/mL, plasma Hsp90 $\alpha$  showed a sensitivity of 93% and specificity of 91% in detecting cancer patients. Similar results were noted for early-stage liver cancer (sensitivity 91%, specificity 91%).  $\alpha$ -Fetoprotein (AFP) is a well-accepted tumor biomarker for the diagnosis of HCC. However, its sensitivity is only 25-65% at the

commonly used cutoff value of 20 ng/mL [41]. Due to its low sensitivity, the 2010 American Association for the Study of Liver Diseases (AASLD) guidelines has cancelled the use of AFP as a screening indicator for HCC [42]. However, plasma Hsp90a measurement showed much more precise in distinguishing AFPnegative HCC patients (AUC 0.971, sensitivity 94%, specificity 91%) and AFP-limited liver cancer (AUC 0.971, sensitivity 97%, specificity 90%) from non-liver cancer control, including healthy individuals and patients with non-cancerous liver disease [43]. This quantitative ELISA kit of plasma Hsp90a has now been approved by China Food and Drug Administration (CFDA) to be used in lung cancer and liver cancer patients (see http://www.sfda.gov.cn). Also, we are continuing to expand the use of plasma Hsp90a in many other cancer types, including colorectal cancer (AUC 0.955, sensitivity 94%, specificity 87%) and breast cancer (AUC 0.764, sensitivity 69%, specificity 74%) (Data not published). These results have proved that plasma Hsp90a is a novel cancer biomarker with a broad spectrum. About 90% of cancer indications, estimated by the World Health Organization, could be hopefully cured if diagnosed at early stage [44]. The detection of plasma Hsp90a represents an early, convenient, accurate, and fast (e-CAF) "liquid biopsy" means for cancer diagnosis, particularly for patients at early stage. In addition, plasma Hsp90a can also be used to monitor the efficacy of cancer therapy [27,39,43]. A biomarker reflecting the dynamic changes responding to the patients' condition can provide the important clinical guidance for doctors. In lung cancer patients, we observed a statistically significant difference between before and after operation, and patients in PD and PR/SD groups [39]. In liver cancer patients, the difference of mean concentration of plasma Hsp90a was also significant between pre and postoperative, and between two interventional therapy groups [43].

It is well known that multidrug resistance, the underlying mechanisms by which many cancers acquired the drug resistance to chemotherapy or targeted therapy, contribute majorly the failure for cancer treatments [45]. Increasing researches have reported that exogenous anti-angiogenesis drugs can induce the tumor metastasis [46-48]. Recently, we also reported that specific chemotherapeutic agents, such as paclitaxel and carboplatin, can promote tumor metastasis through upregulation of the serum levels of cytokine and angiogenic factors, including CXCR2, CXCR4, S1P/S1PR1, PIGF, PDGF-BB, CXCL1 [49]. Therefore, the potent anti-tumor drug with low toxicity and no drug resistance is urgently needed [50,51]. Endostatin, a 20-kDa C-terminal proteolytic fragment of collagen XVIII, was first identified as a potent endogenous angiogenesis inhibitor on endothelial cell migration, proliferation, and angiogenesis [50,52-54]. More than 20 years' extensive studies in our lab have unraveled many mysteries of endostatin, including acid-induced unfolding mechanism [55,56]; contribution of disulfide bonds to the structure stability and biological functions [57]; the antitumor effect of nonnative endostatin [58-60]; contribution of zinc ion to the structure stability and biological function [61]; contribution of the N-terminal integrity to the structure stability and biological functions [62]; the mechanisms of internalization and translocation of endostatin [63-70]; establishment of criteria to assure correct folding of endostatin [71] (For more details, see refs [71,72]). On these ground breaking discoveries, recombinant human endostatin was correctly refolded and approved by CFDA to be used for the treatment of non-small cell lung cancer (see http://www.sfda.gov.cn). Further studies have revealed that the fraction of nucleonin on the surface of endothelial cells serves as the functional receptor of endostatin, which mediates the antiangiogenic and anti-lymphangiogenic activities of endostatin [64,66].

Vascular endothelial growth factor (VEGF) and nonmuscle myosin heavy chain 9 (MyH9) cooperatively mobilize nucleolin from nucleus to cell surface [63]. Surface nucleolin can be internalized by two pathways: caveolae/lipid rafts and clathrin-coated pits [65]. Voltagedependent anion channel 1 also mediates the apoptosis function of endostatin on endothelial cells [73]. The identified novel roles for nucleolin provide the fundamental implications for understanding the biology of endostatin and for its personalized application for cancer treatment with endostatin [74].

Our recent findings on endostatin are also extremely exciting. We have found that endostatin can prevent dietary-induced obesity by inhibiting adipogenesis and angiogenesis through interaction with Sam68 RNA-binding protein to paralyzing the mTOR pathway, which revealed that endostatin has a potential application for antiobesity therapy and prevention of obesity-related metabolic syndromes [75]. Another study in our lab reported for the first time that endostatin embedded novel ATPase activity, which mediates its antiangiogenic and antitumor activities [76]. The endostatin mutant with enhance ATPase can significantly inhibit the recruitment and activation of macrophages in non-small cell lung cancer [77]. Endostatin can also chemosensitize p53-deficient non-small cell lung cancer to genotoxic drug by targeting DNA-dependent protein kinase [78]. These studies provide a new direction for more potent antitumor drug development and clinical applications of endostatin.

To control or cure cancer is a systematic process. Here we summarized our strategy that trinity of nucleolin guide, endostatin treatment, and plasma Hsp90 $\alpha$  monitored, for cancer prevention. In clinic, we recommend first detecting the expression levels of nucleolin on the endothelial cell surface of cancer blood vessels of patients before choosing to use endostatin or other drugs for cancer treatment, and finally monitoring the treatment efficacy by measuring plasma Hsp90 $\alpha$ .

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