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The Role of Glycoprotein 96 in Breast Cancer

Uloga glikoproteina 96 u karcinomu dojke

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Abstract. Glycoprotein 96 (gp96) is a member of the heat shock protein 90 family, which is an ubiquitous family of molecular chaperones that are involved in the regulation of protein folding and other essential cellular activities. Residing in the lumen of the endoplasmic reticulum, gp96 plays a key role in maintaining protein homeostasis, from assemblage to degradation. However, exposure to stressful conditions that disturb cellular homeostasis may translocate gp96 to the cell surface, which implies its additional functions, such as the regulation of intracellular signalling, proliferation, and apoptosis, as well as the modulation of the immune response. Besides its roles under physiological conditions, gp96 is also included in different stages of oncogenesis. In this review, we summarised available data on the structure, physiological, and pathophysiological roles of gp96, particularly in breast cancer oncogenesis.

Keywords: Breast Neoplasms; Carcinogenesis; Cell Proliferation; HSP90 Heat-Shock Proteins; Prognosis

Sažetak. Glikoprotein 96 (gp96) član je obitelji proteina toplinskog šoka 90 koja je inače sveprisutna obitelj molekularnih šaperona uključenih u regulaciju sinteze proteina, ali i drugih esencijalnih staničnih procesa. Gp96 je smješten u lumenu endoplazmatskog retikula stanice gdje igra ključnu ulogu u homeostazi proteina, od njihove sinteze do razlaganja. Međutim, uslijed izloženosti stresornim čimbenicima koji dovode do narušavanja stanične ravnoteže, može doći do premještanja gp96 na staničnu membranu pri čemu se aktiviraju njegove dodatne funkcije, kao što su regulacija unutarstanične signalizacije, proliferacije, apoptoze te modulacija imunološkog odgovora. Pored njegove uloge u fiziološkim uvjetima, gp96 također ima i aktivnu ulogu u različitim fazama onkogeneze. U ovom preglednom članku objedinili smo dostupna saznanja o strukturi, fiziološkim te patofiziološkim ulogama gp96, prvenstveno onima u onkogenezi kod karcinoma dojke.

Ključne riječi: karcinogeneza; karcinom dojke; prognoza; proteini toplinskog šoka 90; stanična proliferacija

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INTRODUCTION

Over the past several decades, heat shock proteins (HSPs) have garnered considerable attention in the field of scientific research. They play an important role in regulating various cellular life activities such as maintaining protein homeostasis, transport within the cell structure, developmental processes, and immune response, as well as tumour proliferation, angiogenesis, and metastasis. This large family of proteins is categorised into the following groups regarding their molecular weight: HSP27, HSP40, HSP60, HSP70, HSP90, and large HSPs. The HSP90 family has five members encoded by the HSPC gene family, from HSPC1 to HSPC5, and each member has its own unique array of client proteins and biological roles that markedly affect cellular signalling cascades. Their client protein network accounts for up to several hundred proteins. The HSP90 family, along with the HSP70 family, is considered one of the most widely studied groups of HSPs[1, 2].

STRUCTURE AND PHYSIOLOGICAL ROLES OF GP96

Glycoprotein 96 (gp96), also known as glucose-regulated protein 94 (GRP94), HSP90B1, or endoplasmin, is the main chaperone of the HSP90 family, which resides in the endoplasmic reticulum (ER). Its molecular weight is around 94–100 kDa, and it is a typical „V" shaped homodimer containing four structural domains. The N-terminal domain contains a binding site for nucleotides, peptides, CNPY3 (the cochaperone that regulates toll-like receptors' folding and export), sites for the interaction with dendritic cells whilst presenting antigens, and a putative transmembrane domain. The M-terminal domain is responsible for ATP hydrolysis, while the C-terminal domain mediates gp96 dimerization and ER localization through the KDEL sequence. The charged linker is the fourth domain that connects the N- and M-terminal domains and is important for nucleotide and calcium binding[3].

Gp96 expression is upregulated by interferons and different environmental stressors such as hypoglycemia, acidosis, ultraviolet (UV) irradiation, oxidative stress, microbial infection, inflammation, and accumulation of misfolded proteins[4]. It has a complex role in various physiological functions. Many studies have shown that gp96 serves as a master immune chaperone, important for both innate and adaptive immune system functioning. It is essential in the process of early lymphopoiesis of B- and T-lymphocytes, the suppressive function of regulatory T cells (Tregs), as well as for macrophages' role in the innate immune defence. According to the literature, it is also important for the folding of toll-like receptors (TLRs), integrins, Wnt coreceptor low-density lipoprotein receptor-related protein 6 (LRP6), glycoprotein A repetitions predominant (GARP), platelet glycoprotein Ib/IX/V complex, and insulin-like growth factor, which all have been found to participate at different phases of cancer development, demonstrating that gp96 is essential in oncogenesis[5–7].

In the tumour microenvironment, gp96 transfers to the cell surface, increasing tumour immunogenicity and activating the anti-tumour immune response.

THE BIOLOGICAL ROLE OF GP96 IN CANCER

Gp96 primarily resides in the lumen of the endoplasmic reticulum. However, in the aforementioned stressful environment, it can translocate to the cell surface. Studies have shown that the cell surface expression of gp96 is important for dendritic cell maturation, promotion of antigen presentation and activation, and differentiation of Th2 cells. However, gp96 is also found in various cancer cells, including multiple myeloma, hepatocellular cancer, lung cancer, breast cancer (BC), oesophageal cancer, and gastric cancer[8]. As a stress chaperone, gp96 is important in the control of pro-oncogenic signalling pathways and cell adhesion, as shown in different mouse models[9]. In the tumour microenvironment, gp96 also transfers to the cell surface, increasing tumour immunogenicity and activating the anti-tumour immune response[10, 11]. These data suggest that gp96 is a promising target for cancer immunotherapy, and thus far there have been several studies about gp96-based tumour vaccination,

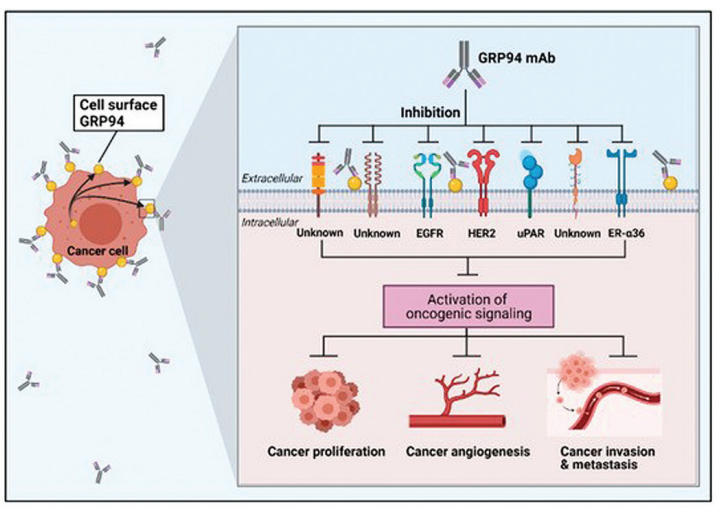

Figure 1. Illustration of the monoclonal antibody targeting gp96 (GRP94) and its inhibitory effect on cancer cells. Gp96 is overexpressed on the surface of cancer cells and plays a crucial role in a major client network, acting as a chaperone for proteins such as HER2, uPAR, ER-α36, EGFR, and many others. However, numerous proteins that interact with gp96 remain unidentified. The binding of gp96 with its client proteins activates oncogenic signaling and promotes cancer development. By targeting the cell surface gp96, monoclonal antibodies can hinder these interactions, ultimately impeding cancer cell proliferation, invasion, and metastasis. HER2 – human epidermal growth factor receptor 2; uPAR – urokinase-type plasminogen activator receptor; ER-α36 – estrogen receptor α36; EGFR – epidermal growth factor receptor. Adapted from Kim JW, Cho YB, Lee S. Cell Surface GRP94 as a Novel Emerging Therapeutic Target for Monoclonal Antibody Cancer Therapy. Cells 2021;17:10:670 (CC BY 4.0). © 2021 Ji Woong Kim, Yea Bin Cho, Sukmook Lee.

The inhibition of gp96 on a cell membrane with monoclonal antibodies leads to the suppression of HER2-driven cell growth and an increase of apoptosis, suggesting that gp96 could be a potential target in the treatment of HER2-positive BC.

monoclonal antibodies against gp96, and selective gp96 small molecule inhibitors[8] (Figure 1).

THE ROLE OF GP96 IN BREAST CANCER

BC cells have higher expression of gp96 compared to normal mammary tissue[12]. In addition, cell surface expression of gp96 on BC cells depends on the expression of oestrogen and progesterone receptors (ER, PR), human epidermal growth factor receptor 2 (HER2), and the mitotic proliferation index Ki-67. Radolović et al. found a higher expression of gp96 in triple-negative BC compared to luminal A and luminal B HER2-negative molecular subtypes, as well as a higher expression of gp96 in patients with local recurrence of HER2 enriched BC. It also affects CD4+ and CD8+ T-lymphocytes in different molecular BC subtypes[13]. Accordingly, it is considered a biomarker for immune surveillance and immune invasion, possibly via exosomes, small extracellular vesicles used in intercellular communication[14, 15]. Gp96 plays an important role in the activation of HER2 receptors on the cell membrane and their downstream signalling pathways[16]. Patel et al. found that gp96 inhibition using paralog-selective HSP90 inhibitors induces apoptosis and impairs cell viability in HER2-overexpressing BC cell lines. They confirmed that gp96, along with other HSP90 paralogs, regulates distinct HER2 functions on the cell membrane and plays an important role in the cytosolic pathways of HER2 species[17]. Li et al. found that gp96 and HER2 form a complex on the cell membrane and that gp96 overexpression in HER2-positive BC cell lines significantly improves HER2 dimerization, phosphorylation, and downstream signalling. The inhibition of gp96 on a cell membrane with monoclonal antibodies leads to the suppression of HER2-driven cell growth and an increase of apoptosis, suggesting that gp96 could be a potential target in the treatment of HER2-positive BC[18]. Recent studies showed that a high expression of gp96 in BC cells correlates with aggressive BC molecular subtypes[19, 20], chemoresistance, poorer survival[21, 22] and brain metastasis progression. Martinez-Aranda et al. found that both FN14 and gp96 are useful biomarkers for early risk stratification of brain metastasis[23]. Gp96 also positively regulates ER-α36 expression, which is important for BC growth and the development of tamoxifen resistance. By blocking the interaction between gp96 and ER-α36, Hou et al. managed to suppress BC cell proliferation and invasion[24]. Buc Calderon et al. investigated, in their experimental model with BC cell lines, the influence of gp96 on tumour aggressiveness, its influence on BC cells' resistance against doxorubicin, as well as the influence of the tumour microenvironment on the regulation of overall expression of gp96. They found that the inhibition of gp96 expression using siRNA against gp96 decreased the invasive-

ness of BC cells and increased the sensitivity to doxorubicin. The overall expression of gp96 in different BC cell lines remained unchanged in the settings of hypoxia, chemical-like induced hypoxia, and normal conditions. They confirmed that the expression of gp96 is controlled by glucose depletion[19]. According to the research of Dejeans et al., the overexpression of gp96 in BC promotes cancer cell migration and proliferation while also conferring resistance to oxidative stress. They also found a significantly higher expression of gp96 in the recurrence compared to the corresponding primary BC, but the latter was not due to unfolded protein response activation. These findings suggest that gp96 overexpression might be a key mechanism in deriving cancer cells to more aggressive phenotypes following chemoresistance[20]. By examining gp96 expression in BC tissue and its relationship with clinicopathological features, Liu et al. found an increased cytoplasmic expression of gp96 that was negatively correlated with the expression of PR. However, gp96 expression did not correlate with age, tumour size, lymph node metastasis, clinical stage of the disease, or the expression of ER. Using molecular methods, they also found that gp96 mRNA was significantly higher in BC cells compared to normal breast tissue. Along with common clinicopathological features that affect survival in BC, they confirmed that a higher gp96 expression correlates with a worse prognosis and that gp96 expression can be used as an independent prognostic factor for survival in BC patients[21]. According to Cawthorn et al., a higher gp96 expression is associated with the development of distant metastases, as well as with worse overall and disease-free survival[25]. However, a recent study that examined the gene expression profiles and prognostic significance of several HSP90 family members in BC revealed that, despite increased gene expression levels, only the expression of HSP90AA1 was associated with tumour stage and survival[26].

CONCLUSION

Despite advances in cancer research, the way tumours develop resistance to available standard therapies is still poorly understood. Therefore, the latter issue is a leading challenge to successful cancer treatment. Since cancer cells have substantial genomic and phenotypic variations, the identification of the factors that serve as predictors of poorer survival, more aggressive phenotypes, and tumour resistance to conventional therapies is of critical relevance for the improvement of diagnostic and treatment strategies and the development of future therapeutics. In this review, we discussed the importance of gp96 in normal cell functioning, as well as its fundamental role in different phases of oncogenesis and tumour aggressiveness. Current knowledge points to the potential that gp96 may have as a target for anti-tumour therapy, which could prevent metastatic dissemination or achieve therapeutic effects in patients with disease progression due to resistance to tumour therapy.

Conflicts of Interest: Authors declare no conflicts of interest.

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