GARP and Tumor Immunosuppressive Microenvironment

Subjects: Oncology

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Tumors are not only composed of cancer cells but also of various infiltrating cells constituting the tumor microenvironment (TME); all these cells produce growth factors which contribute to tumor progression and invasiveness. Among them, TGF-β, in particular the predominant isoform, TGF-β1, plays a major role in tumor progression due to its pleiotropic effects (1). TGF-β is, in fact, a potent immunosuppressive cytokine, impacting antitumor immune responses (2) and it has many other protumor effects related to its role in epithelial–mesenchymal transition, cell proliferation, cell invasion and angiogenesis (3,4) and resistance to treatments (5,6). However, its use as a biomarker is made difficult by the existence of several inactive forms upstream of the biologically active TGF-β. Glycoprotein-A repetition predominant (GARP) is the docking receptor for latent transforming growth factor (LTGF-β) and promotes its activation. Increased GARP expression has been found in many types of cancer. GARP is expressed by regulatory T cells and platelets in the tumor microenvironment (TME) and can be also expressed by tumor cells themselves. Thus, GARP can be widely present in tumors in which it plays a major role in the production of active TGF-β, contributing to immune evasion and cancer progression via the GARP-TGF-β axis.

The objective of this review is to highlight GARP's expression and function in cancer and to evaluate its potential as a predictive and therapeutic follow-up biomarker that could be assessed, in real time, by molecular imaging.

Keywords: GARP ; TGF-β ; cancer ; biomarker ; immunosuppression

1. Overview

Tumors are not only composed of cancer cells but also of various infiltrating cells constituting the tumor microenvironment (TME); all these cells produce growth factors which contribute to tumor progression and invasiveness. Among them, TGF-β, in particular the predominant isoform, TGF-β1, plays a major role in tumor progression due to its pleiotropic effects[1]. TGF-β is, in fact, a potent immunosuppressive cytokine, impacting antitumor immune responses[2] and it has many other protumor effects related to its role in epithelial–mesenchymal transition, cell proliferation, cell invasion and angiogenesis[3][4] and resistance to treatments[5][6]. However, its use as a biomarker is made difficult by the existence of several inactive forms upstream of the biologically active TGF-β. Glycoprotein-A repetition predominant (GARP) is the docking receptor for latent transforming growth factor (LTGF-β) and promotes its activation. Increased GARP expression has been found in many types of cancer. GARP is expressed by regulatory T cells and platelets in the tumor microenvironment (TME) and can be also expressed by tumor cells themselves. Thus, GARP can be widely present in tumors in which it plays a major role in the production of active TGF-β, contributing to immune evasion and cancer progression via the GARP-TGF-β axis.

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2. GARP Expression, Structure and Function

2.1. GARP Expression

The GARP gene is expressed in various tissues[7] and in different cell types, such as Tregs and activated B lymphocytes, megakaryocytes and platelets, mesenchymal stromal cells (MSC), hepatic stellate cells and human umbilical vein endothelial cells[8][9]. Although the GARP gene is detected in many cell types, the expression of the GARP protein has been reported only at the membrane of Tregs[10][11], activated B cells[12], platelets[8][11][13], MSC[9] and hepatic stellate cells[14]. GARP membrane expression has been shown to be dependent on the endoplasmic reticulum (ER) stress protein Gp96[15]. In cancer, an amplification of GARP gene, as well as GARP expression, has been found in tumor cells, particularly in invasive, metastatic or treatment-resistant tumors[16][17][18].

2.2. GARP Structure

GARP is a 72 kDa type I transmembrane protein consisting of 662 amino acids. Its structure consists of three regions: the extracellular domain, with leucine-rich repeats, accounting for about 70% of the protein; the hydrophobic transmembrane domain; and a cytoplasmic tail of 15 amino-acid residues[7][19]. The extracellular portion of GARP contains 20 LRR motifs, divided into two groups by a proline-rich region, and a C-terminal LRR. Two cysteines (positions 192 and 331, 7th and 12th LRR, respectively), are responsible for two disulfide bonds between GARP and LAP in LTGF-β[1][20].

2.3. GARP Promotes the Activation of Biologically Active TGF-β

GARP is the docking receptor for LTGF-β[11][21][22][23][24][25] and promotes its activation[20][21][25]. TGF-β is expressed in its latent form: latent-TGF-β (LTGF-β), to prevent the binding of active TGF-β to its receptor[26]. LTGF-β can then associate with GARP which allows its surface expression[10]. GARP can then orient LTGF-β for binding to integrin αVβ8 or αVβ6[20][23] via an arginine-glycine-aspartate (RGD) motif, allowing the further release of the biologically active TGF-β from LTGF-β through a protease-dependent—or independent—mechanism. An increased expression of GARP has been shown to increase the bioactivity of TGF-β and cause oncogenesis[1].

3. GARP in Cancer

GARP can be widely present in a tumor, both on tumor cells and on cells of the TME. By positively regulating TGF-β in the TME, GARP promotes oncogenesis. Moreover, GARP can be secreted (soluble GARP).

3.1 GARP and Cancer Cells

GARP is widely expressed by human cancer cells compared with normal epithelial cells[27]. GARP may support cancer cell growth and dissemination by providing a reservoir of TGF-b that functions in the TME by suppressing the innate and adaptive immune responses, inducing extracellular matrix deposition, invasion, loss of cellular adhesion, metastasis formation and angiogenesis.

3.2 GARP and Cells of the TME

GARP is found to be highly expressed on the surface of activated Tregs and to maintain their regulatory functions[28]. Infiltration of Tregs expressing GARP in the TME is associated with poor prognosis in various types of cancer, including melanoma[27], lung[29], colon[30] and gastric[31] cancers. Indeed, Tregs have been shown to inhibit antitumor immune responses, thus enhancing tumor progression[27][29][30][31].

Platelets have been also found to constitutively express GARP with increased expression upon platelet activation[13]. Coagulation and platelet activation may contribute to immune evasion and cancer progression via the GARP-TGF-β pathway.

3.3 Soluble GARP

Studies indicate the existence of soluble GARP[7][32]. Soluble GARP plays a role in the modulation of T-cell function and can influence the polarization of macrophages[27].

4. GARP as a Therapeutic Target in Cancer

The role of GARP in the production of the active form of TGF-β and in Tregs homeostasis makes it an interesting target in some cancers[25].

Two anti-GARP monoclonal antibodies (mAbs) that block the production of active TGF-β by human Tregs have been reported to inhibit human Tregs *in vitro* and *in vivo* and overcome resistance to anti-PD-1 therapy in tumor-bearing mice[33]. These results make blocking anti-GARP-TGF-β interaction using mAbs an interesting approach to treat patients with cancer resistant to currently available immunotherapies (like immune checkpoint inhibitors).

In addition, the specific deletion of GARP in platelets has been shown to inhibit TGF-β signaling, thus promoting anti-tumor immunity in various cancer types[13]. These findings highlight a novel therapeutic strategy in cancer based on the combination of GARP inhibition with platelet inhibitors.

5. GARP as a Biomarker in Cancer: Perspectives

Regarding its expression on tumor cells and on immunosuppressive cells of the TME, as well as its function in TGF-β activation, GARP may represent an interesting biomarker. GARP has been shown to be overexpressed in different cancers and may represent a prognosis biomarker. However, the correlation between its expression and the different stages of these cancers remains to be demonstrated taking in account treatment information. Indeed, advanced stages are most often under treatment, which could impact GARP expression. Indeed, it has been shown that neoadjuvant chemotherapy decreased the infiltration of GARP+ Tregs in intratumoral gastric cancer[31] and suggested that GARP could therefore represent a marker for therapeutic monitoring and response to treatment. In addition, the implantation of tumor cells with enhanced GARP expression led to increased tumor growth, as well as resistance to chemotherapy and radiotherapy. Thus, GARP might also serve as a predictive marker of resistance to treatment[34]. In this context, *in vivo* molecular imaging (e.g., positron emission tomography, PET) targeting GARP could then represent an interesting tool. However, to our knowledge, there is currently no data on GARP imaging in cancer. This approach could allow *in vivo* phenotyping and monitoring of the tumor to determine its aggressiveness, treatment options and responses, in a noninvasive, rapid and personalized manner.

6. Conclusions

GARP is the docking receptor for LTGF-β and promotes its activation. In cancer, increased GARP expression has been shown in many cancers of bad prognosis, both in tumor cells and in cells of the TME, where it plays a major role in the production of active TGF-β, thus contributing to the immunosuppressive environment. Regarding its expression as well as its function via TGF-β activation, GARP may represent an interesting biomarker for prognosis and therapeutic follow-up. In vivo molecular imaging, such as PET and targeting GARP, could represent an interesting, personalized approach to further investigate GARP potential as such a biomarker in cancer.

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