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Mini-Review: GARP, a Putative Potential Molecule in Tumor Immunosuppressive Environment

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ABSTRACT

Glycoprotein A Repetitions Predominant (GARP), also known as leucine-rich repeats containing 32 (LRRC32), is a transmembrane protein that presents latent TGF-β1 on the surface of regulatory T cells (Tregs) and modulates its activation in tumor immunosuppressive environment. Tregs are immunosuppressive immune cells that play an important role in tumor development and progression. Inhibition of Treg function is considered to be an effective strategy for antitumor therapy. In addition to its expression in Tregs, GARP has been recently found to be highly expressed in a few types of human solid tumor tissues, yet the role of its expression in tumor tissues or cells remains unknown. Most previous studies on GARP have focused on GARP function in Tregs and the role of GARP in latent TGF-β1 activation. The present review provides an up to date overview of GARP expression and its potential role in tumor cells and tissues.

Introduction

Regulatory T cells (Tregs) are a small population of CD4+ T lymphocytes and play an important role in tumor development and progression[1]. Activation or enhancement of Treg function is one of the main mechanisms by which cancer evades antitumor immune attacks through a variety of different pathways. Inhibition of Treg function is also considered to be an effective antitumor treatment strategy. Previous studies have shown a significant association of expression of Treg specific markers (Foxp3, and CD39) with lymph node metastasis and poor prognosis[2-4]. However further studies of Tregs is limited by a of lack of available markers for activated Tregs. Although Foxp3 is very important in the development and function of Tregs[5], it is expressed intracellularly in both Tregs and conventional T cells[6-8], making it challenging to isolate and further characterize Tregs. Specific markers for activated Tregs are therefore necessary for research in the specific mechanisms of Tregs. Glycoprotein A Repetitions Predominant (GARP), also known as leucine-rich repeats containing 32 (LRRC32), has been widely accepted to be a marker of activated Tregs, which contribute to an immunosuppressive tumor microenvironment[9,10]. It has been demonstrated that GARP is the cell surface docking receptor for latent TGF-β and exerts its antitumor function, at least in part, by activating TGF-β in tumor microenvironment[11].

TGF-β and GARP

Transforming growth factor-β family members (TGF-β1, TGF-β2

and TGF-β3) are pleiotropic cytokines expressed by most cells and are found in all tissues. They play a key role in cell proliferation, differentiation, migration, invasion and immune function, etc[12]. Dysregulations in TGF-β function are associated with a variety of pathological conditions including cancer. TGF-β2 and TGF-β3 are mainly involved in embryonic development. TGF-β1 has been extensively studied in regulating the immune response. Biochemically, TGF-β exists in at least four different forms: (1) freely soluble TGF-β; (2) soluble TGF-β associated with latency-associated peptide (LAP), known as latent TGF-β (LTGF-β); (3) TGF-β-LAP-LTBP, latent TGF-β associated with latent TGF-β-binding protein (LTBP); and (4) membrane-associated latent form of TGF-β[13,14]. Only TGF-β without LAP is known to be biologically active. GARP is highly expressed on the surface of activated Tregs and the overexpression of GARP increases the inhibitory function of Tregs[15]. GARP can bind to latent TGF-β and promote secretion and activation of TGF-β1[16,17]. Additionally, GARP protein is also found to be expressed on human tumor cells where it likely mediates the accumulation and subsequent activation of latent TGF-β[11]. By regulating innate and adaptive immune components and facilitating tumor immune evasion, GARP provides an excellent TGF-β reservoir that plays a role in the tumor microenvironment, thereby supporting the growth and progression of cancer cells. In terms of innate immunity, TGF-β inhibits the maturation of natural killer (NK) cells and dendritic (DC) cells[18,19]. Furthermore, TGF-β impairs the adaptive antitumor immunity by directly inhibiting the clonal expansion and cytotoxicity of the CD8+ cytotoxic T cells(CTLs)[20,21]. Finally, TGF-β indirectly attenuates CTLs by inducing the expression of Foxp3, which confers a regulatory and immune suppressive phenotype to CD4+ T cells[22].

GARP Gene and Expression

The GARP-encoding gene LRRC32 was firstly identified as DI1S863E in the human 11q13.5-q14 chromosomal region and defined on chromosome 7 in the region 7E-7F in mice[23,24]. LRRC32 gene is frequently amplified in breast cancer[9,10]. It consists of two encoding exons and is expressed at two major transcripts of 4.4 and 2.8 kb. The first exon encodes the signal peptide and nine amino acids, and the second exon encodes most of the coding region[25]. GARP gene is expressed in a variety of tissues including placenta, lung, kidney, heart, liver, skeletal muscle, pancreas and lymphoid tissues[10]. In addition, GARP gene is detected in multiple cell types such as megakaryocytes, platelets[26,27], Tregs[15,28], endothelial cells[29,30] and hepatic stellate cells[31], as well as embryonic stem cells and fibroblasts[32]. Single nucleotide polymorphisms (SNP) located in the noncoding regions of human GARP are associated with higher risks of developing diseases, including certain types of cancers[33,34]. GARP expression is also regulated by microRNAs. MiR-142-3p, miR-181a, miR-185, miR-24, and miR-335 are thought to bind to the 3' UTR of GARP to repress its expression[35,36]. Thereafter, miR-142-3p represses posttranscriptional regulation of GARP expression by Argonaute 2-associated degradation of GARP mRNA. The Argonaute protein family plays a central role in RNA silencing processes, as essential components of the RNA-induced silencing complex (RISC). Argonaute 2 binds miR-142-3p, which guides Argonaute 2 to the 3' untranslated region of GARP through sequence complementarity, which then leads to GARP mRNA cleavage or translation inhibition. Downregulation of miRNA, in turn, may be one way to induce GARP expression in Tregs.

GARP Protein and Expression

GARP is an 80kD transmembrane protein consisting of 662 amino acids. Its structure can be divided into three regions: the extracellular domain with leucine-rich repeats, accounting for about 70% of the protein; the hydrophobic transmembrane domain; and cytoplasmic tail of 15 amino acid residues[10,25]. Tregs frequently accumulate in the tumor tissues and peripheral blood of patients with cancer. Their increased frequency has generally been considered to be the marker of poor cancer prognosis, presumably due to the Treg-mediated antitumour immunosuppression[1]. GARP is co-expressed with latent TGF-β on the surface of activated Tregs, but not on other types of T cells and is thus regarded as a specific marker of activated Tregs[16,37,38]. In one study, as a TGF-β docking receptor, the number of GARP+Foxp3+ Tregs was found to be significantly higher in patients with advanced hepatocellular carcinoma than in the control group. Furthermore, the GARP expression levels of Foxp3+ Tregs were elevated in these patients[39]. Compared with the control group, the expression of GARP in Tregs was increased in tumor tissues of lung cancer patients and was associated with lymph node metastasis, distant metastasis and clinical stage[40]. These study findings indicate that increased expression of GARP promotes cancer progression through activation of immunosuppressive functions of Tregs. More interestingly, in a clinical study of advanced gastric cancer, the tumor tissue infiltration of Foxp3+ and GARP+Tregs in the neoadjuvant chemotherapy group was significantly lower than that of the control group[41]. Monoclonal antibodies against GARP in GARP/TGF-β1complexes not only blocked the production of active TGF-β1 by human Tregs but also inhibited the immunosuppressive activity of human Tregs in a mouse model of Xenogeneic graft -versus-host disease[42]. One study however reported that only deletion of GARP in mouse Tregs was not sufficient to impair their immunosuppressive function in vivo[43].

GARP Protein in Cancer Tissues and Cancerous Cell Lines

As a novel marker of activated Tregs, GARP has been

extensively studied; most of studies were focused on Tregs from peripheral blood or cancerous tissues as noted above. Increased GARP protein expression has only been found in a few types of cancers, such as breast, lung, and colon cancers[11], where the increase in GARP expression was significantly correlated with advanced stage and poor prognosis. Enforced expression of GARP in normal murine mammary cells upregulated TGF-β bioactivity and drove oncogenesis. More importantly, treatment of mice transplanted with a murine tumor expressing GARP with anti-GARP antibody decreased the number of Tregs in the blood, and inhibited lung metastasis. Concomitant treatment of chemotherapy and anti-GARP antibody inhibited primary tumor growth. While these results demonstrate the promising function of GARP in tumor treatment, the role or function of GARP expressed by tumor cells is still unclear. Little has been reported about the role of GARP expressed in cancer cells. Interestingly, Hahn et al[44] found that GARP was not only expressed in activated Tregs but also in melanoma cells. More importantly, sGARP (soluble GARP) shed from both cell types had similar inhibitory properties which contributed to an immunosuppressive tumor environment by influencing the phenotype and function of monocytic-myeloid lineage cells, inhibiting CD4+ and CD8+ effector T cell functions and inducing Tregs. These findings reveal a new mechanism in which GARP expression on cancer cells may be a novel molecule that contributes to the tumor immunosuppressive environment. To continue this line of research, our research team[45] recently found a significant increase in the expression of GARP in papillary thyroid carcinoma (PTC) compared with benign thyroid diseases (including nodular goiter and adenoma). Compared to the benign thyroid diseases elevated GARP expression in PTC was positively correlated with increased expression of Foxp3, which is very important for the development of Tregs. However, there was no significant association of elevated expression of GARP with lymph node metastasis in PTC. This study demonstrated the potential role of GARP in the pathogenesis of PTC. Our results suggest that GARP might be a novel antitumor therapeutic target. Moreover, our research further supports the existence of a positive feedback loop between GARP and Foxp3 (Figure 1). It has been also found GARP protein expression in some cell lines such as some melanoma cell lines[44].

Both GARP and Foxp3 are important markers of Tregs, but the relationship between them is still controversial. Some studies have shown that GARP and Foxp3 may form a positive feedback loop. Retroviral overexpression of GARP in antigen-specific Th cells resulted in an efficient and stable re-programming of effector T cells towards Tregs, which involved up-regulation of Foxp3. Lentiviral downregulation of GARP in Tregs significantly impaired the suppressor function and was associated with downregulation of Foxp3. Moreover, the downregulation of Foxp3 resulted in similar phenotypic changes and down-regulation of GARP[37,46]. GARP overexpression in naïve T cells induced the expression of Foxp3. Silencing Foxp3 in human Tregs reduced expression of GARP[15]. In our study, the positive correlation between increased GARP and elevated Foxp3 expression provides further evidence for the existence of this loop[45]. In contrast, there are some studies that did not support the existence of a positive feedback loop between GARP and Foxp3[16,47,48]. However, further studies are needed to explore the relationship between GARP and Foxp3.

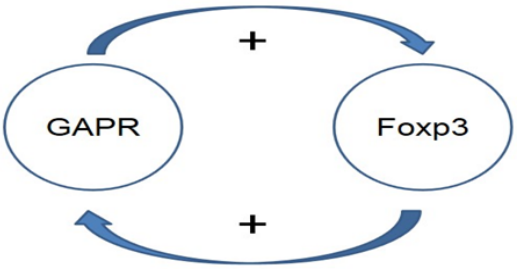

Figure 1: GARP and Foxp3 forms a positive feedback loop.
Some studies have shown that increased GARP leads to an increase in Foxp3, and an increase in Foxp3 also leads to an increase in GARP, vice versa.

Conclusion and Future Perspectives

GARP is a docking receptor for latent TGF-β and is involved in its activation. The function of GARP-TGF-β in Treg biology has been a topic of increasing interest. More recently, the tolerogenic role of GARP expression by cancer cells has gained attention due to the possibility that GARP could be an attractive target for antitumor immunotherapy. Moreover, since GARP is not only expressed by activated Tregs but also by some types of tumor cells, it may be an ideal target for a combinatory tumor immune therapy and serve as a novel checkpoint in future therapeutic strategies.

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