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Role of microRNAs on HLA-G expression in human tumors

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ABSTRACT

The non-classical human leukocyte antigen G (HLA-G) known to protect the embryo from immune cell destruction leading to fetal maternal tolerance is often overexpressed in human tumors of distinct origin thereby leading to an escape from T and NK cell-mediated immune response. The molecular mechanisms controlling HLA-G expression are complex and involve deregulation at the transcriptional, epigenetic and posttranscriptional level. Using bioinformatics and high through put analyses a number of microRNAs (miRs) have been identified, which were able to bind to the 3′ UTR of HLA-G with distinct efficacy. This caused by a downregulation of HLA-G surface expression, which was associated with an increased immune response thereby overcoming the HLA-G-mediated immune tolerance. Reduced expression of HLA-G-specific miRs was associated with tumor progression and metastases and appear to affect directly or indirectly tumor characteristics, such as cell proliferation, apoptosis and resistance to chemotherapy. Recently, an interaction between long non-coding RNAs, such as HOTAIR, and HLA-G-specific miRs has also been demonstrated. This review summarizes the control of HLA-G expression and function by microRNAs as well as its clinical significance.

1. Physiologic and pathophysiologic features of HLA-G

The non-classical human leukocyte antigen (HLA)-G exerts well-described immune modulatory activities, such as downregulation of the innate and adaptive immune responses as well as induction of tolerance due to its interaction with multiple inhibitory receptors present on T and B lymphocytes, natural killer (NK) cells and antigen presenting cells (APC) including ILT2/LILRB1, ILT4/LILRB2 and KIR2DL4 [1–3] as well as with CD160 on endothelial cells. Under physiological conditions HLA-G is expressed at high levels in immune-privileged organs, like cornea, thymus, pancreatic islets and at the fetal maternal interface on trophoblasts, but only at marginal or low levels in most adult cells.

However, HLA-G expression can be induced under pathophysiological conditions, in particular during malignant transformation, viral infections, inflammation and auto-immune diseases [4–7]. Using immunohistochemistry (IHC), Western blot and/or qPCR analyses an increased, but highly variable HLA-G expression has been detected in many solid tumors of distinct histology [8–12] including solid tumors, e.g. hepatocellular carcinoma (HCC), renal cell carcinoma (RCC), neuroblastoma, breast, colorectal and lung as well as malignancies, such as acute myeloid leukemia (AML) and B cell chronic lymphocytic leukemia [13–15]. Interestingly, HLA-G mRNA expression of tumors is more frequent than that of the corresponding protein expression. The discordant mRNA and protein expression levels suggested a post-transcriptional regulation of HLA-G. It is noteworthy that some tumors not only express HLA-G on the cell surface, but also secrete soluble HLA-G (sHLA-G) as shown for e.g. HCC, RCC, melanoma, breast and lung cancer as well as AML. The aberrant expression levels and/or secretion of HLA-G had clinical relevance and are often associated with disease progression, metastasis and poor outcome of tumor patients [16].

Abbreviations: AML, acute myeloid leukemia; APC, antigen presenting cells; CTL, cytotoxic T lymphocyte; DAC, 5-aza-2′-deoxycytidine; DNMT1, DNA methyltransferase 1; HCC, hepatocellular carcinoma; IFN, interferon; IHC, immunohistochemistry; lnRNA, long non-coding RNA; MHC, human major histocompatibility complex; miRs, microRNAs; MMP, matrix metalloproteinases; NK, natural killer; RBP, RNA-binding proteins; RCC, renal cell carcinoma; sHLA-G, soluble HLA-G; SNP, single nucleotide polymorphism; TME, tumor microenvironment; Treg, regulatory T cells; 5′-URR, 5′ upstream regulatory region; 3′-UTR, 3′ untranslated region.

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2. Molecular mechanisms of aberrant HLA-G expression in tumors

The underlying molecular mechanisms of aberrant HLA-G expression in human tumors have been investigated in detail and appear to be diverse (Fig. 1). In contrast to classical HLA class I antigens mutations and/or deletions of HLA-G have not been described. In addition, the HLA-G locus shows a lower polymorphism with some genetic variations both in the 5′ upstream regulatory region (URR) and the 3′ untranslated region (3′-UTR). Some polymorphic sites in the 3′ UTR of HLA-G interfere with the mRNA stability, alternative splicing and binding of specific microRNAs (miRs) thereby modulating HLA-G mRNA and/or protein expression. The HLA-G polymorphisms highly varied in their frequency and differed in distinct ethnic populations [17–19] and has been shown to be of predictive and prognostic value.

A meta-analysis of breast cancer lesions demonstrated that the frequency of the 14 base pair (bp) insertion/deletion polymorphism was associated with an overall risk to develop breast cancer [17,18], HCC [20] or bladder cancer in smoking patients. Although only a limited number of tumor types and ethnic groups have been investigated, some of the polymorphisms and allelic variations of the 3′-UTR of HLA-G were associated with the tumor development in distinct ethnic populations [8]. These data suggested that polymorphisms in these regions play an important role in the control of mRNA and protein levels, and may influence mRNA stability and binding of microRNAs (miRs).

In addition, HLA-G expression could be transcriptionally regulated by external factors and soluble mediators, such as cytokines, growth factors, hormones, stress, nutrient deprivation, immune suppressive drugs and hypoxia [4,21–23]. Another possibility of regulating HLA-G expression are epigenetic mechanisms, such as DNA methylation and histone acetylation. Indeed, lack of HLA-G expression in tumors has been shown to be due to methylation of the CpG islands in the HLA-G promoter [24,25]. Treatment in particular with the demethylating 2′5′ agent 5-aza-2′-deoxycytidine (DAC), but also with the histone deacetylase inhibitor trichostatin A was able to reactivate HLA-G gene and protein expression in malignant cells *in vitro* [25,26]. In most cases HLA-G expression is enhanced rather than *de novo* induced by these agents [22]. Recently, also a post-transcriptional control of HLA-G mediated by miRs has been suggested.

3. Characteristics of miRs and their role in tumors

MiRs represent small, single-stranded, non-coding RNAs of 17–24 nucleotides in length, which control the post-transcriptional gene expression by binding to the 3′ UTR of mRNA of target genes thereby affecting their stability and/or translation [27]. So far, more than 2500 human miRs have been identified controlling at least 50% of all protein-coding genes in mammalians [28,29]. miRs exert pleiotropic effects and play an important role in many physiologic and pathophysiologic processes. They have been shown to be involved in the regulation of the innate and adaptive immune responses as well as in the initiation and progression of tumors [30,31]. High throughput analysis demonstrated global changes within the miRNA expression pattern in normal when compared to tumor tissues. Interestingly, the miR genes were frequently located at fragile sites and cancer-associated chromosomal regions [32]. The deregulated expression of miRs, but also of enzymes involved in miR biogenesis is involved in the initiation as well as progression of tumors, metastasis formation and therapy resistance [30,33]. Furthermore, miRs can participate in reprogramming components of the tissue tumor microenvironment (TME) in order to promote tumorigenicity [34].

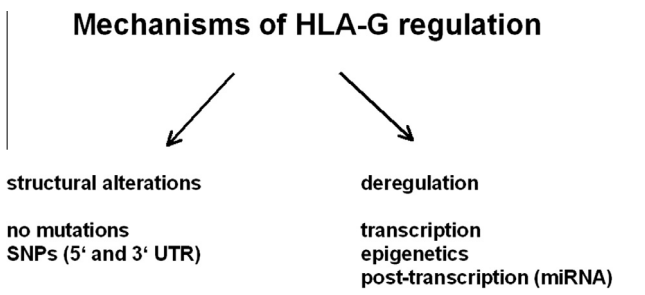

Fig. 1. Mechanisms of regulation of HLA-G expression. The mechanisms of the control HLA-G of HLA-G expression are summarized.

In the following section, miRs identified as powerful regulators of HLA-G involved in escape from immune surveillance and their clinical relevance are described suggesting that these HLA-G-specific miRs might serve as putative targets for (immune) therapy.

4. HLA-G-regulating miRs, their expression and function *in vitro* and *in vivo*

Different strategies including *in silico* analyses, miR arrays and RNA sequencing lead to the identification of a number of HLA-G-regulating candidate miRs [9,35–39] (Fig. 2) including members of the miR-152 family, such as miR-148a, miR-148b, miR-152, but also miR-133, miR-139-5p, miR-548, miR-608 and miR-628. For some of these candidate miRs binding to the 3′-UTR has been shown and their abundance could be categorized as miR-152 > miR-148A > miR-148B > miR-133 using both luciferase reporter assays and miTRAP [9,40] and appear to depend on the sequence of the HLA-G 3′ UTR. For example miR-139-3p could bind to non-polymorphic sequences of the HLA-G 3′-UTR in a stable and specific manner, while others, such as miR-608, bind to polymorphic sequences and therefore the binding of miRs might be influenced by the variant [37]. This hypothesis was further confirmed by the distinct binding affinity of miR-152 to the HLA-G 3′-UTR in RCC cell lines caused by the presence of different HLA-G alleles [9].

Overexpression of these miRs had also functional activities, since these miRs could directly downregulate HLA-G mRNA and/or surface expression. Although only a few studies *in vitro* and *in situ* studies were performed correlating HLA-G and HLA-G-specific miR expression, an inverse association of both HLA-G and HLA-G-specific miRs was found: the miR-152 family members are expressed at low levels in the placenta compared with other healthy tissues [36,41], which is consistent with the specific high expression levels of HLA-G observed in the placenta [36]. In RCC cell lines an inverse expression of HLA-G and HLA-G-specific miRs has been described [9]. These data were further confirmed by IHC of RCC lesions demonstrating an inverse correlation between miR-148a and HLA-G, but not for the HLA-G-specific miR-152 and miR-148b [9]. The discordant expression of miR-148a and HLA-G expression was of clinical significance, since it was associated with tumor grading and staging. The HLA-G-specific miR-mediated inhibition of HLA-G expression has functional relevance by modulating immune responses. High levels of HLA-G-specific miR expression restored recognition by immune cells leading to an increased NK cell-mediated cytotoxicity [9].

The inverse correlation of HLA-G and HLA-G-specific miRs in tumors might be due to their epigenetic control [42–44]. Not only methylation of the HLA-G promoter, but also of miR-148a has been described [41]. Due to hypermethylation of its CpG island, miR-148a undergoes a methylation-mediated silencing in tumor cells. Since the DNA methyltransferase DNMT1 maintaining methylation