The Role of MicroRNAs in the Control of Innate Immune Response in Cancer

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Ligands for receptors of natural killer (NK) cells and CD8+ cytotoxic T lymphocytes (CTL), such as the inhibitory nonclassical HLA-G, the activating stress-induced major histocompatibility complex class I-related antigens MICA and MICB, and/or the UL16-binding proteins (ULBPs), are often aberrantly expressed upon viral infection and neoplastic transformation, thereby preventing virus-infected or malignant-transformed cells from elimination by immune effector cells. Recently, it has been shown that ligands of both NK and CD8+ T cells are regulated by a number of cellular and/or viral microRNAs (miRs). These miRs are involved in shaping the antiviral and/or antitumoral immune responses as well as neoplastic growth properties. This review summarizes the expression pattern and function of miRs directed against selected NK and T cell receptor ligands, their putative role in shaping immune surveillance and tumorigenicity, and their clinical relevance. In addition, the potential role of RNA-binding proteins in the post-transcriptional gene regulation of these ligands will be discussed.


microRNAs: Their Characteristics and Functions

microRNAs (miRs) represent a large family of about 20 nt long, noncoding, single-stranded RNA molecules, which are transcribed by RNA polymerase II and processed from long primary transcripts (pri-miRs) by the nuclear RNase III Drosha. The resulting precursor miRs (pre-miRs) are exported from the nucleus to the cytoplasm by the protein exportin-5 and processed to the mature miRs by the RNase III Dicer (1,2).

The mature miRs are integrated into an RNA-induced silencing complex (RISC) and act as translational repressors upon sequence-specific binding to target messenger RNAs (mRNAs) preferentially at the 3’ untranslated region (3’-UTR). In silico analyses as well as biologic evidence demonstrate that the miR-mediated gene regulation represents a fundamental mechanism of post-transcriptional control by modulating more than 50% of all cellular genes in mammals (3,4).

However, the identification of miR targets is a complicated task, because 1) miR sequences do not fully match with their target mRNA; 2) the molecular mechanisms controlling miR and mRNA interactions are not yet completely understood; and 3) the in silico algorithms developed to predict miR targets are very inaccurate with a specificity often lower than 70% (5).

The expression and activity of miRs could be modified by both intrinsic and extrinsic factors, such as chromosomal alterations, epigenetic modifications, polymorphic promoter elements, and even single nucleotide polymorphisms (SNPs), eg, if localized within the miR binding site at the target mRNA. Recently, disease-associated SNPs at miR binding sites affecting the post-transcriptional gene regulation have been identified (6–9).

Regarding their function, miRs can exert either tumor suppressor or oncogenic activity, which often correlates with relevant tumor characteristics, such as cell proliferation, apoptosis, differentiation, migration, invasion, metastasis formation, tumor initiation, and disease progression (10). Furthermore, miRs have been shown to be important in the development and modulation of the immune cell repertoire, as well as of the innate and adaptive immune responses (11,12). The link between miR and function of the immune system has recently been analyzed, in particular for T cells. miRs have been identified to regulate T cell response, maturation, differentiation and function, such as activation, proliferation and apoptosis (13). These include, for example, the oncogenic members of the miR-17–92 cluster involved in apoptosis of CD4+ T cells (14), while miR-222 and miR-339 promote resistance of cancer cells to cytotoxic T lymphocytes (CTL) by downregulation of ICAM-1 (15).

Elimination of Tumor Cells by the Innate and Adaptive Immune System

Developing tumors could be eliminated by both innate and adaptive immune cells (16). NK cells, which represent a subset of the innate immune system, are known to eliminate tumors either lacking the expression of classical HLA class I molecules or expressing stress-induced activating NK cell receptor ligands. Nevertheless, tumor cells expressing both classical HLA class I and nonclassical HLA class I antigens (eg, HLA-G), are more resistant to NK cell-mediated lysis (17). The cytotoxic NK cell activity can be modulated by a balance of activating and inhibitory signals sensed via respective receptor ligand interactions (Table 1) (18–20). Inhibitory NK
<table>
<thead>
<tr>
<th>Receptor</th>
<th>NK cell receptor</th>
<th>Chromosomal localization</th>
<th>Ligand(s)</th>
<th>Chromosomal localization</th>
<th>Length of 3'-UTR</th>
<th>No. of in silico predicted miRs (<a href="http://www.mirdb.org">www.mirdb.org</a>)</th>
<th>Published miRs</th>
<th>No. of in silico predicted RBPs (rbpdb.ccbr.utoronto.ca/)</th>
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<td><strong>NK cell activating receptors</strong></td>
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<td>NKp30</td>
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<td>B7H6</td>
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<td>4806 nt</td>
<td>76</td>
<td>-</td>
<td>-</td>
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<td>AICL</td>
<td>12p13-p12</td>
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<td>35</td>
<td>-</td>
<td>-</td>
<td>22</td>
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<td>NKR2B4</td>
<td>1q23</td>
<td>CD48</td>
<td>1q21-q22</td>
<td>690 nt</td>
<td>16</td>
<td>-</td>
<td>-</td>
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<td>12p13-p12</td>
<td>MICA</td>
<td>6p21</td>
<td>174 nt</td>
<td>14</td>
<td>miR-17-5p/-20a/-93 (68); miR-520b (76)</td>
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<td></td>
<td></td>
<td>MICB</td>
<td>6p21</td>
<td>1229 nt</td>
<td>32</td>
<td>miR-10b (79), miR-373/-376a/-433/BART2-5p (EBV)/UL112 (HCMV)/K12-7 (KSHV) (71,72)</td>
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<td>ULBP1</td>
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<td>miR-34a/c (69)</td>
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<td>ULBP3</td>
<td>6q25</td>
<td>230 nt (67)</td>
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<td>BKV-miR-B1-3p/JCV-miR-J1-3p (67)</td>
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<td></td>
<td>ULBP5</td>
<td>6q24-q25</td>
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<td>-</td>
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<td>ULBP6</td>
<td>6q25</td>
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<td></td>
<td></td>
<td>LETAL</td>
<td>6q25</td>
<td>Unknown</td>
<td>-</td>
<td>-</td>
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<td>1q23</td>
<td>IgGs</td>
<td>14q32</td>
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<td>DNAM-1</td>
<td>18q22</td>
<td>CD112</td>
<td>19q13</td>
<td>862 nt</td>
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<td></td>
<td></td>
<td>CD155</td>
<td>19q13</td>
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<td>19q13</td>
<td>4351 nt</td>
<td>-</td>
<td>28</td>
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<td>NTB-A (upon NK cells)</td>
<td>1q23</td>
<td>NTB-A (upon CD4+ T cells)</td>
<td>1q23</td>
<td>1685 nt</td>
<td>-</td>
<td>23</td>
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<td></td>
<td></td>
<td>CRACC</td>
<td>1q23-24</td>
<td>Unknown</td>
<td>-</td>
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<td>21</td>
<td></td>
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<td></td>
<td>NKG2C/CD94</td>
<td>12p13/12p13</td>
<td>HLA-E</td>
<td>6p21</td>
<td>1476 nt</td>
<td>-</td>
<td>28</td>
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<td></td>
<td></td>
<td>NKG2E/CD94</td>
<td>12p13/12p13</td>
<td>HLA-E</td>
<td>6p21</td>
<td>1476 nt</td>
<td>-</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activating KIRs</td>
<td>19q13</td>
<td>Mostly unknown</td>
<td>-</td>
<td>-</td>
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<td></td>
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<tr>
<td>LILRB1</td>
<td>19q13</td>
<td>HLA-G</td>
<td>6p21</td>
<td>383 nt</td>
<td>26</td>
<td>miR-133a/-148A/-148B/-152 (78,79)</td>
<td>23</td>
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<td>LILRB2</td>
<td>19q13</td>
<td>HLA-C</td>
<td>6p21</td>
<td>420 nt</td>
<td>3</td>
<td>miR-148 family (80,81)</td>
<td>21</td>
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</table>

*3'-UTR = 3' untranslated region; EBV = Epstein Barr virus; HCMV = human cytomegalovirus; HLA = human leukocyte antigen; KSHV = Kaposi's sarcoma associated herpesvirus; miR = microRNA; NK = natural killer; nt = nucleotides; RBP = RNA-binding protein.
cell receptors monitor the presence or absence of classical and non-classical HLA class I antigens leading to an increased susceptibility of HLA-negative tumor cells to NK cell–mediated lysis because of their failure to deliver inhibitory signals to NK cells (21,22). The cytotoxicity of NK cells is further controlled by the interaction of the activating NK cell receptor NKG2D with its ligands.

In addition, tumor cells could also be eradicated by CD8+ cytotoxic T lymphocytes (CTL), which recognize tumor antigens presented in the context of HLA class I molecules. Therefore, a lack of HLA class Ia expression leads to tolerance of tumor cells against recognition by CTL. Furthermore, the T and NK cell– mediated antitumoral immune responses could be shaped by alterations of the tumor microenvironment, in particular by the presence of immune suppressive cells, soluble mediators, like cytokines, eg, the secretion of immunosuppressive factors like the IL-10, TGF-β, and adenosine, as well as other factors, such as pH and hypoxia (23–26).

Features of the Activating NK Cell Receptor NKG2D

The NK cell receptor NKG2D is a C-type lectin-like, type II transmembrane glycoprotein, which is constitutively expressed in human NK, NKT, macrophages, and γδ T cells, as well as in a subset of CD8+ T cells, where it serves as a costimulatory receptor (27). Engagement of NKG2D on NK and T cells could lead to the killing of pathogen-infected target cells as well as tumor cells. For example, in different murine models, rejection of transplanted tumors and a reduced incidence of spontaneous tumors were associated with enhanced NKG2D-mediated cytotoxicity (28). Many factors have shown to inhibit or enhance NKG2D surface expression. These include the adaptor molecules DAP10 and DAP12, as well as various cytokines, such as IL-2, IL-7, IL-12, and IL-15, which increase the NKG2D expression, whereas IL-21, IFN-γ, and TGF-β decrease its expression (29).

Properties of the NKG2D Ligands MICA, MICB, and ULBPs: Their Expression Pattern and Regulation

NKG2D binds to multiple ligands (NKG2D-L), such as the major histocompatibility complex class I-related molecules (MIC) A and B and the human cytomegalovirus (HCMV) UL16-binding proteins (ULBP) 1–6, also known as RAET1 proteins and LETAL (Figure 1; Table 1) (30–35). Members of the MIC and ULBP family are phylogenetically distinct, exert sequence divergence and vary in their domain architecture (36). With the exception of the nervous system, MICA and MICB transcripts were found in tissues of healthy individuals, ULBPs were expressed in various tissues like heart, lung, and liver (37,38), the NKG2D-L LETAL is expressed in a variety of normal tissues, eg, brain, breast, and colon (31). The surface expression of NKG2D-L is highly regulated in order to avoid inappropriate immune responses (39) and could be induced by various stress situations, such as viral infection, oxidative stress, genotoxic drugs, tissue damage, heat shock, inflammatory cytokines, and malignant transformation (31,33,35,36,40,41).

Although the molecular mechanisms regulating the NKG2D-L expression are not completely understood, NKG2D-L expression could be controlled at the transcriptional and post-transcriptional levels, including cleavage from the cell membrane by proteases (35). In addition, genes involved in the malignant and viral transformation process could also modulate NKG2D-L expression, like HER-2/neu and E1A, which enhance the expression of MICA and MICB (42,43). In this context, it is noteworthy that the loss of the tumor suppressor p53 as well as the expression of the oncogenes

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The NKG2D receptor and its activating ligands, grouped for their protein structure and their known expression profiles (eg, exempli gratia) (31,73,207–210). NK = natural killer.
RAS, myc, or the protein kinase AKT did not affect NKG2D-L expression (35). Next to it, the ATM/ATR DNA damage repair pathway active in early tumorigenesis could affect NKG2D-L expression: Treatment of cells with DNA-damaging agents, such as ionizing radiation, UV-C, and cisplatin, as well as histone deacetylase (HDAC) inhibitors, replication inhibitors, the proteasome inhibitor bortezomib, retinoic acid, and glycogen synthase kinase-3, increased the expression of single or multiple NKG2D-L. Other cellular pathways activated during viral infection or malignant transformation like signalling via the toll-like receptors (TLR)-4 and TLR-7/8 or cytokine receptors could also induce a differential transcription of NKG2D-L: IFN-γ and TGF-β down-regulate MICA and ULBP expression in melanoma and glioblastoma cells (44), while IFN-α treatment of dendritic cells (DC) and of tumor cells enhanced MICA expression (45). For LETAL, an upregulation by TNF-α was detected, whereas other inflammatory cytokines, hypoxia or starvation had marginal to no effect on NKG2D-L expression (31).

Thus, the NKG2D-L regulation is very complex and is mediated at distinct levels by various signals from stressed, infected or transformed cells. Multiple checkpoints operating at distinct levels of NKG2D-L expression might not only facilitate their expression, but might also fine-tune their expression kinetics. However, the rapid induction of NKG2D-L on the cell surface of tumor and virus-infected cells is essential and ensures rapid elimination of these cells.

**MICA, MICB, and ULBP Expression in Tumor Cells and Its Clinical Significance**

Many tumor cell lines and primary tumor lesions of distinct tissue origin express single or various NKG2D-L, but their expression is heterogeneous regarding the quality and quantity of respective ligands. A high frequency of MIC expression was found within several primary tumors, including leukemia, breast, kidney, colorectal, prostate, hepatocellular, and carcinoma and melanoma. Human ULBPs are also expressed by primary tumor cells, including breast cancer, ovarian cancer, hepatocellular carcinoma, melanoma, and acute myeloid leukemia (Table 2) (29).

In situ studies using immunohistochemical analysis of tumor lesions in order to correlate the expression of NKG2D-L with clinicopathological parameters revealed in some cases contradictory results. Expression of the NKG2D-L was often associated with a better prognosis. The frequency and levels of MICA expression are reduced in metastatic melanoma lesions when compared with primary tumors, suggesting that their loss is associated with tumor progression. Similar results were obtained in colorectal carcinoma, in which high levels of MICA expression were associated with a good prognosis of tumor patients (46,47). In B cell chronic lymphatic leukemia, soluble—but not membrane-anchored—MIC expression could be linked to clinical parameters (46).

The secretion and/or shedding of NKG2D-L from tumor cells could lead to a downregulation of NKG2D on NK and T cells, resulting in decreased cytotoxicity of these immune effector cells. It is noteworthy that not only sMICA, but also sULBP1–3, could affect NK cell activity, although a functional impact of sNKG2D-L on immune responses has not consistently been observed (48). However, sMICA/B and sULBP1–3 found in sera of cancer patients were correlated with poor prognosis (48–52), suggesting that they might represent potential therapeutic targets (29).

**Characteristics of HLA-G Expression, Its Regulation and Function**

HLA-G is a nonclassical HLA class Ib molecule that possesses unique features when compared with classical HLA class Ia antigens, including a low polymorphism, restricted and tightly controlled expression, and the existence of seven splice variants, including four membrane-bound and three soluble isosforms (53–55). A high degree of variation exists in its promoter region, which could influence the binding of specific transcription factors (enhancers or repressors), thereby modulating its expression.

<table>
<thead>
<tr>
<th>Tumor entity</th>
<th>Cohort size</th>
<th>Method</th>
<th>MICA</th>
<th>MICB</th>
<th>ULBP1</th>
<th>ULBP2</th>
<th>ULBP3</th>
<th>ULBP4</th>
<th>ULBP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer (199)</td>
<td>677</td>
<td>IHC</td>
<td>50</td>
<td>90</td>
<td>99</td>
<td>100</td>
<td>26</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma (200)</td>
<td>54</td>
<td>IHC</td>
<td>Up</td>
<td>59</td>
<td>0</td>
<td>0</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>Medulloblastoma (201)</td>
<td>54</td>
<td>IHC</td>
<td>98</td>
<td>n.d.</td>
<td>n.d.</td>
<td>8</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>Melanoma (51)</td>
<td>16</td>
<td>IHC</td>
<td>75</td>
<td>n.d.</td>
<td>n.d.</td>
<td>50</td>
<td>n.d.</td>
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<tr>
<td>Neuroblastoma (202)</td>
<td>17</td>
<td>IHC</td>
<td>0</td>
<td>n.d.</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>n.d.</td>
<td></td>
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<tr>
<td>Ovarian cancer (203–205)</td>
<td>442</td>
<td>IHC</td>
<td>42</td>
<td>n.d.</td>
<td>14</td>
<td>32</td>
<td>13</td>
<td>15</td>
<td>19</td>
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<tr>
<td>Sezary syndrome (lymphoma) (206)</td>
<td>10</td>
<td>Flow cytometry</td>
<td>20</td>
<td>10</td>
<td>20</td>
<td>60</td>
<td>60</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* The expression of ULBP6 and LETAL has not yet been analyzed on in vivo tumor material. NKG2D-L expression (%). IHC = immunohistochemistry; n.d. = not determined.

Table 2. Summary of all clinical studies investigating the expression profile of the activating NKG2D ligands (protein level) in various tumor entities*
In addition, in the 3'-UTR region of HLA-G, different polymorphic sites have been identified, which appear to be potentially associated with the magnitude of HLA-G expression. These include the 14 bp insertion/deletion polymorphisms and two single nucleotide polymorphisms (SNP) (56,57).

The physiologic, constitutive expression of HLA-G is mainly restricted to embryonic and immune-privileged tissues, as well as trophoblasts of the maternal placenta. Aberrant HLA-G expression is often associated with pregnancy complications, such as preeclampsia, and upon transformation processes (58,59). HLA-G inhibits NK cell activity via direct interaction with the inhibitory leukocytic receptor LILRB1, which has a higher affinity to HLA-G than to other HLA class I molecules. This interaction provides tolerance at the fetomaternal interface. HLA-G can also bind to the inhibitory receptors LILRB2 and KIR2DL4 (Table 1) (60,61).

**HLA-G Expression in Tumors and Its Clinical Relevance**

A number of studies have shown that HLA-G is often expressed in solid and hematopoietic tumors. These include among others melanoma, renal cell carcinoma, colorectal cancer, ovarian carcinoma, pancreatic adenocarcinoma, and T cell lymphomas (56,58). However, the frequency and levels of HLA-G expression strongly varied, suggesting a distinct HLA-G expression pattern in tumors. In addition, HLA-G expression is also found on tumor-infiltrating cells (62). This pathophysiologic expression of HLA-G provides immune protection of the tumor cells from NK and CD8+ T cell-mediated cytotoxicity. Furthermore, trogocytosis mediating the intercell transfer of viable HLA-G molecules renders CD8+ CTL unresponsive to tumor antigens (63,64). This is in accordance with high levels of membrane-bound and soluble HLA-G expression, which were often associated with reduced antitumoral immune responses, disease progression, and poor clinical outcome of tumor patients (65,66).

**miRs Regulating NKG2D-L Expression**

Recent reports have provided evidence that NKG2D-L expression could be regulated by distinct cellular and viral miRs targeting MICA, MICB, and/or ULBPs, respectively (67–72,74–76). Based on in silico analysis, miR arrays, and functional studies, 14 cellular and five viral miRs have been identified to target the 3'-UTRs of MICA, MICB, and ULBP1-3 (Table 1). The targeting of MICA, MICB, and ULBP1-3 by miRs caused not only a reduced-surface expression of these proteins, but also an evasion of malignant or viral-infected cells from the NKG2D-mediated elimination by NK cells. The three different viral miRs (miR-UL112 [HCMV], miRBART2-5p [EBV], and miR-K12-7 [KSHV]), all derived from herpesviridae, selectively target MICB, while other stress-induced NKG2D-L are not affected. In contrast, one miR with identical sequence in the JCV and BKV (BKV-miR-B1-3p/JCV-miR-J1-3p) viruses targets ULBP3. So far, it is not understood why viral miRs target only one stress-induced ligand. One possible explanation might be the evolution of the 3'-UTRs avoiding equal sequences, thereby preventing multiple stress-induced ligands to be targeted by just one viral miR. Alternatively, it is possible that the viruses target stress-induced ligands that are specifically induced during infection.

Interestingly, several of the cellular miRs, eg, miR-17-5p/20a/93/10b/650, targeting NKG2D-L have an oncogenic potential (Table 3), but not all miRs known to downregulate NKG2D-L are oncogenic. The expression of some MICA/B-targeting miRs could be modulated by immune stimuli, suggesting that the composition of the tumor microenvironment could also affect miR expression and function in tumor cells. Lipopolysaccharides (LPS) decreased the expression of miR-17-5p, miR-20a, and miR-93, which target MICA in macrophages (68). In addition, cytokines, in particular IFN-γ, elevated the expression of miRs controlling MIC expression (76).

In the case of the ULBPs, only ULBP1 and two specific cellular miRs have been found. ULBP2 expression is controlled in melanoma by tumor-suppressive miRs of the miR-34 family. The members of this family, miR-34a and miR-34b/c, directly affect the translation of ULPB2, while decreased miR-34 expression levels upregulate this protein (69). Interestingly, the expression of miR-34a and miR-34b/c is controlled by the tumor suppressor protein p53, further linking miR regulation of stress-induced ligand expression to tumor development (77).

**Regulation of HLA-G by miRs**

So far, many different mechanisms have been described to control HLA-G expression (58). These include its transcriptional downregulation by promoter hypermethylation, transcriptional repression, and post-transcriptional gene regulation. In addition, the 14 base pair (bp) insertion/deletion polymorphism in the 3'-UTR of HLA-G, has been associated with RNA stability. Furthermore, the discordant mRNA and protein expression was detected in many tumor cells and in virus-infected cells, suggesting a post-transcriptional gene regulation of HLA-G, which might be mediated by miRs.

Indeed, different miRs have been recently identified that target the 3'-UTR of HLA-G, like members of the miR-148 family: miR-148a, miR-148b, and miR-152 (78), as well as miR-133a (79). miR-152 and miR-148a/b overexpression resulted in a reduced HLA-G expression by directly targeting the 3'-UTR in HLA-G+ cells. This has functional consequences, because miR-152 and miR-148a/b were able to restore the HLA-G-mediated inhibition of T and NK cell cytotoxicity. In this context it is noteworthy that the miR-148 family also impairs HLA-C expression, the inhibitory ligand for KIR2DL1-3 on NK cells (Table 1) (80,81).

Furthermore, next to miRs regulated by viral and malignant transformation thereby affecting HLA-G expression, other miRs, eg, miR-133a, have recently been identified to decrease HLA-G protein in a distinct pathophysiologic context. This miR-133a-mediated downregulation of HLA-G did correlate with the induction of spontaneous abortions, suggesting that miR-controlled HLA-G expression could be also involved in pregnancy complications (79).

Recently, monitoring the role of sHLA-G on the miR expression profile of T cells demonstrated an altered miR expression pattern in activated sHLA-G-treated vs untreated CD4+ T cells: While miR-210 expression was increased, miR-451 expression was...
<table>
<thead>
<tr>
<th>miR</th>
<th>NK cell receptor ligand target</th>
<th>Biological impact of the miR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proliferation</td>
</tr>
<tr>
<td>miR-20a</td>
<td></td>
<td>Down (87)</td>
</tr>
<tr>
<td>miR-93</td>
<td></td>
<td>Down (119–121)</td>
</tr>
<tr>
<td>miR-520b</td>
<td></td>
<td>Down (123–125)</td>
</tr>
<tr>
<td>miR-10b</td>
<td>MICB</td>
<td>Up</td>
</tr>
<tr>
<td>miR-140-5p</td>
<td>ULBP1</td>
<td>Down</td>
</tr>
<tr>
<td>miR-409-3p</td>
<td></td>
<td>Down (126)</td>
</tr>
<tr>
<td>miR-650</td>
<td></td>
<td>Up (5, 130)</td>
</tr>
<tr>
<td>miR-34c</td>
<td></td>
<td>Down (144, 155, 175–178)</td>
</tr>
<tr>
<td>miR-133a</td>
<td>HLA-G</td>
<td>Down (88–91, 93, 96, 183–185)</td>
</tr>
<tr>
<td>miR-148A</td>
<td>HLA-C, HLA-G</td>
<td>Down</td>
</tr>
<tr>
<td>miR-148B</td>
<td>HLA-G</td>
<td>Down (191–196)</td>
</tr>
<tr>
<td>miR-152</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* HLA = human leukocyte antigen; miR = microRNA; n.d. = not determined.
decreased in the presence of sHLA-G, suggesting that sHLA-G is able to tune the miR expression of T cells, thereby shaping the antitumoral immune responses (82).

**Novel Putative Mechanisms of NKG2D-L and HLA-G Regulation: RNA-Binding Proteins and Usage of Alternative Poly(A) Sites**

Despite the fact that different cellular and viral miRs play a crucial role in the post-transcriptional gene regulation of HLA-G, MICs, and ULBPs, other cellular processes might also control their expression. In this context, RNA-binding proteins (RBPs) recently identified to be overexpressed in various cancer types might be also involved in the regulation of HLA-G or NKG2D-L (83). Although such RBPs have not yet been identified, in silico analysis predicts 23 different candidate RBPs possibly binding to the HLA-G mRNA (Table 1).

Another molecular mechanism of shaping NKG2D-L or HLA-G expression is represented by shortening of the 3′-UTRs using alternative poly(A) sites, which might affect their translation (84). Interestingly, different NKR ligands exert highly variable numbers of possible poly(A) sites, which appear to be independent of the 3′-UTR length of the NKR ligand (Table 4). While ULBP1 exerts 20 possible poly(A) sites, only four poly(A) sites were found in HLA-G. It could be also speculated that the NKG2D-L– or HLA-G–specific 3′-UTR is shortened under various conditions (Mandelboim, personal communication). Thus, alternative 3′-UTRs might have regulatory potential for at least some of the ligands in tumors; this requires further investigations.

**The Dual Role of NKG2D-L– and HLA-G–Specific miRs**

Because one miR could target multiple mRNAs, one could speculate that the different miRs identified to modulate NKG2D-L and HLA-G might also affect the transformed immunogenicity and enhancing tumor growth and migration. So far, there exists limited information concerning the effect of the immunomodulatory and tumorigenic effects of NKG2D-L– and HLA-G–specific miRs identified.

The miR-10b-mediated downregulation of MICB expression is associated with a reduced sensitivity to NK cell–mediated lysis, while antagonizing of miR-10b not only enhanced the NKG2D-mediated killing of tumor cells in vitro, but also increased tumor clearance in vivo (75). miR-10b has been demonstrated to represent one of the most powerful metastamiRs involved in metastasis formation and disease progression of several tumors, in particular of breast carcinoma. In contrast, the miR-10b antagonist suppressed tumorigenicity because of the restoration of the expression of the transcription factor HOXD10 in vivo (85,86). Thus, inhibition of miR-10b activity during tumor development reduced metastasis formation through two distinct mechanisms: enhanced expression of the transcription factor HOXD10 and boosting the NKG2D-mediated immune attack. In addition, there exists growing evidence that deregulation of miR-520b–suppressing (MICA) contributes to tumorigenesis, because its overexpression was associated with an altered migration of breast cancer cells, as well as impaired growth of hepatoma cells in vitro and in vivo (87). Furthermore, this miR could be induced by IFN-γ, which reduced the MICA surface expression (76), while a miR-520b antagonist increased the expression of MICA.

Concerning miR-133a (known to control HLA-G protein stability) an altered cell cycle progression associated with a reduced proliferative capacity of different tumor cells, and enhanced apoptosis was demonstrated (88–91). Even more interestingly, miR-133a is deregulated in many tumor types of distinct origin, including ovarian (92), prostate (93), bladder (94), and head and neck squamous cell cancer (95). The loss of miR-133a expression was also associated with a poor survival of tumor patients (Table 3) (96).

The miR-148 family members involved in regulating HLA-G also play a functional role in many biologic processes, since they bind to the 3′-UTR of different target genes important for cell growth, development, migration, apoptosis cell cycle progression, migration, and angiogenesis (97). Aberrant expression of miR-148/152 was found in both cancers and nontumor diseases. In tumors, including in hepatocellular carcinoma as well as gastrointestinal cancers, miR-148/152 expression is often decreased, indicating that these miRs act as tumor suppressors. Furthermore, low miR-148a expression levels were strongly associated with lymph node metastases in gastric cancer, while miR-148a overexpression induced suppression of cell proliferation, migration, and invasion in these cells (Table 3).

**Table 4.** Length of the 3′-UTRs and the number of potential poly(A) sites of HLA-G and NKG2D-L with possible consequences for post-transcriptional gene regulation

<table>
<thead>
<tr>
<th>Gene</th>
<th>Length of 3′-UTR in nt</th>
<th>No. of possible poly(A) sites within 3′-UTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-G</td>
<td>383</td>
<td>4</td>
</tr>
<tr>
<td>ULBP1</td>
<td>2382</td>
<td>20</td>
</tr>
<tr>
<td>ULBP2</td>
<td>548</td>
<td>3</td>
</tr>
<tr>
<td>ULBP3</td>
<td>230</td>
<td>0</td>
</tr>
<tr>
<td>ULBP4</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>ULBP5</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>ULBP6</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>MICA</td>
<td>174</td>
<td>1</td>
</tr>
<tr>
<td>MICB</td>
<td>1229</td>
<td>1</td>
</tr>
</tbody>
</table>

* 3′-UTR = 3′ untranslated region; nt = nucleotides
Thus, the identification of distinct miR expression patterns and their effects on NKG2D-L might lead to a comprehensive understanding of the role of these deregulated miRs in tumorigenicity (Figure 2). These miRs do not only tune immune responses, but rather alter the tumor phenotype, suggesting a dual role of these miRs in tumor immune evasion and enhancing tumorigenicity. Because of their distinct function, these miRs might not only be used as potential molecular diagnostic markers, but rather employed as targets for the development of novel therapeutic strategies.

Future Perspectives: Therapeutic Use of miRs Modulating Immune Responses

So far, only a few miRs have been successfully used as therapeutic targets in animal models to inhibit cancer metastasis. HLA-G and NKG2D-L represent attractive targets for therapy that might be broadly used for the treatment of many cancers because of their high frequency of expression on tumor cells. Therapeutic strategies inhibiting HLA-G expression by miRs might be an interesting but also challenging approach, which would then render tumor cells more sensitive to immune cell–mediated recognition. In contrast, in situations in which HLA-G expression is linked with an improved outcome of disease, such as transplantation and pregnancy, HLA-G expression could be enhanced by antagonizing or neutralization of HLA-G–specific miRs. Thus, HLA-G–specific miRs might represent interesting tools to control the immune response in a variety of pathophysiological conditions. Similarly, antagonizing the miRs controlling the stress-induced NKG2D-L expression during virus infection and especially during tumor transformation is also an attractive therapeutic option, because miRs targeting the NKG2D-L are also involved in the tumorigenic process itself (29). Furthermore, NKG2D-L– and HLA-G–specific miRs have an impact on the host/pathogen interaction, including the response to viral infections and neoplastic transformation, because NKG2D-L– and HLA-G–specific miRs could be involved in both cancer suppression and promotion, as well as in host immune responses. The proper delivery of miRs to certain organs remains an essential but difficult issue. Until now, adeno-associated viral particles, as well as chemically-modified antagonomers, have been employed. The latter sustain, in contrast to unmodified antisense oligonucleotides, a long-term effect. Furthermore, a delivery system based on nanoparticles and PEGylation might improve

Figure 2. Role of oncogenic and tumor suppressive miRs for natural killer (NK) cell–mediated cytotoxicity. A) Upregulation of activating NK cell receptor ligands, eg, MICA/B by stressed epithelial cells. B) Tumor cells upregulate oncogenic microRNAs (miRs) and downregulate tumor-suppressive miRs, resulting in the possibility of human leukocyte antigen–G translation (eg, exempli gratia). HLA = human leukocyte antigen; miR = microRNA; NK = natural killer.
the protection of miRs from nuclease digestions. Thus, these miRs represent promising biomarkers and therapeutic targets for various diseases including cancers in the future.

References

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107. Reverting the dysregulated expression of microRNA-10b.


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