OncoImmunology 1:4, 535–536; July 2012; © 2012 Landes Bioscience

Tumor suppressors control ULBP2, an innate surface ligand of the lymphocyte immune receptor NKG2D

Anja Heinemann and Annette Paschen*

Department of Dermatology; University Hospital Essen; Essen, Germany

Keywords: cancer, NKG2D ligand, ULBP2, miR-34, p53

The activating receptor NKG2D, expressed on different innate and adaptive cytotoxic lymphocytes, has been demonstrated to play an important role in anti-tumor immunity. Now evidence is provided that tumor suppressors control expression of its ligand ULBP2 supporting the role of this receptor-ligand system as an innate barrier against tumor development.

Effective anti-tumor immunity is based on the concerted action of innate and adaptive lymphocytes that share expression of the receptor NKG2D.[1] Upon ligand binding on malignant cells NKG2D activates the cytotoxic effector function of Natural Killer (NK) cells and costimulates T-cell activity that in turn leads to the rejection of transplanted tumors and decreases the incidence of spontaneous tumors as shown in different mouse models.[1] Despite the importance of NKG2D in anti-tumor immunity, the mechanisms that control expression of its ligands in malignant cells are poorly understood. In 2005, Gasser et al. demonstrated that genotoxic agents induce NKG2D ligand (NKG2DL) expression in murine and human cells.[2] Based on this observation, the model of NKG2DL as an innate barrier against tumor development emerged which led us to ask for the role of tumor suppressors in the regulation of their expression.

Recently, we demonstrated that different tumor suppressors control expression of ULBP2, a human NKG2DL. Studies on melanoma cell lines revealed that tumor-suppressive microRNAs (miRNAs) repress ULBP2 expression. miRNAs are small non-coding RNAs that regulate mRNA degradation and/or translation primarily by binding to the 3'-untranslated region. We identified members of the miR-34 family (miR-34a and miR-34c) as direct regulators of ULBP2 mRNA, affecting both, its degradation and translation.[3] This miRNA family is of importance for cellular differentiation and when ectopically expressed in malignant cells, induces cell cycle arrest, senescence or apoptosis, explaining its downregulation in many different tumor entities.[4,5] However, some of the melanoma cell lines tested still contained significant amounts of miR-34 that inversely correlated with ULBP2 surface expression. Accordingly, a decrease of miR-34 levels upregulated ULBP2 while an increase of miR-34 downregulated ligand expression that interfered with tumor cell killing by NK cells, demonstrating the functional significance of this regulatory mechanism.[3] As miR-34 family members have recently been characterized for their importance in cellular differentiation, we propose the following model: high levels of miR-34 in differentiated tissues interfere with ULBP2 expression, whereas loss of miR-34, as it frequently occurs in cancer, facilitates ULBP2 protein expression, thereby assisting cytotoxic lymphocytes in immune surveillance of malignant cells.

Interestingly, *MIR34A* and *MIR34B/C* genes are under transcriptional control of the tumor suppressor p53, a regulator activated by various cancer-related stresses including DNA damage.[4] p53 is functionally inactive in more than 50% of cancers and those still containing wild type p53 frequently express elevated levels of the negative regulator MDM2 that by ubiquitination leads to p53 degradation. Different small molecule inhibitors, like Nutlin-3a and RITA, have been developed for cancer therapy that disrupt the MDM2-p53 interaction and induce p53 activation.[6] When we treated p53 wild-type melanoma cells with Nutlin-3a expression of miR-34 was increased. This led to a decrease in ULBP2 levels that could be counteracted by miR-34 specific inhibitors. The same negative effect of Nutlin-3a on ULBP2 was observed also for HCT116 $p53^{+/+}$ colon carcinoma cells but not for isogenic HCT116 $p53^{-/-}$ cells.[3] Thus, Nutlin-3a decreased ULBP2 levels in a p53-dependent manner, which was due, at least in part, to the increase in cellular miR-34 levels (**Fig. 1**).

While our data point to p53 as an indirect negative regulator of ULBP2 expression, recent work by Textor et al. and Li et al. demonstrated that activation of p53 by either ectopic overexpression or

*Correspondence to: Annette Paschen; Email: annette.paschen@uk-essen.de
Submitted: 01/14/12; Accepted: 01/18/12
http://dx.doi.org/10.4161/onci.19406

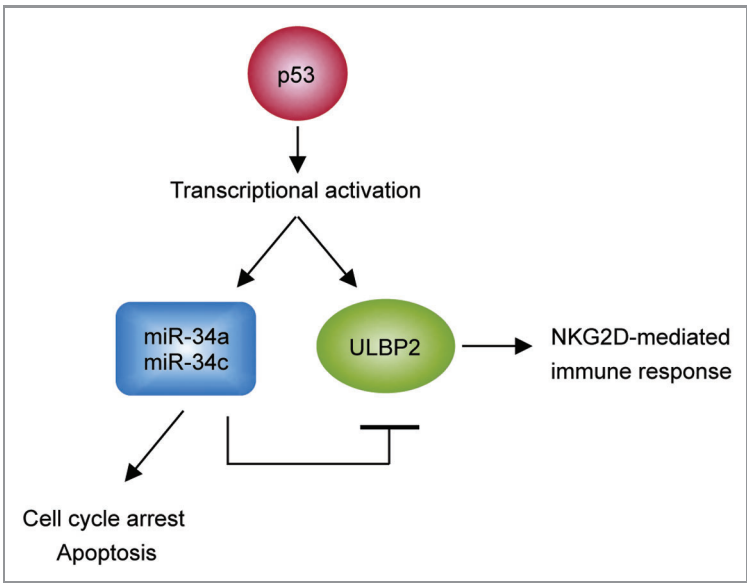

Figure 1. Model of the regulation of ULBP2 expression. Tumor suppressors control expression of the NKG2DL ULBP2 at different levels. The tumor suppressor p53 functions as a direct transcriptional activator of ULBP2. Furthermore it activates transcription of the *MIR34A* and *MIR34B/C* genes. The miR-34 family members miR-34a and miR-34c directly bind to the 3′ untranslated region of ULBP2 mRNA thereby repressing its translation and enhancing its degradation. The balance between both regulatory mechanisms determines the expression level of ULBP2 and in turn the strength of NKG2D dependent immune responses.

treatment of tumor cells with RITA, increased ULBP2 levels via direct enhancement of its transcription (**Fig. 1**).[7,8] Thus, p53 seems to play a dual role in the regulation of ULBP2 expression and we assume that the context of p53 activation determines the outcome. Indeed, it has been demonstrated that Nutlin-3a preferentially induces cell cycle arrest while RITA primarily induces apoptosis in tumor cells, though both substances disrupt the MDM2-p53 interaction. Furthermore the observation by Gasser et al. who found the induction of NKG2DL expression upon DNA damage to be independent of p53 adds another level of complexity to the control of ULBP2 expression.[2]

So far, the involvement of p53 and miR-34 in the regulation of ULBP2 expression suggests that this NKG2DL might be of specific relevance in anti-tumor immunity. Indeed, our previous studies demonstrated that ULBP2 is a marker of exceptional clinical significance in malignant melanoma.[9] Tumor cells have been described to shed NKG2DL by proteolytic cleavage or via exosomes, which allows them to escape from NKG2D-mediated immune responses. Ligand shedding most likely explains the increased levels of soluble NKG2DL found in sera of cancer patients.[1] When measuring patient sera for the presence of soluble ULBP2 and MICA, another NKG2DL expressed on melanoma cells, we detected elevated levels of both markers.[9,10] Interestingly, by correlating soluble MICA (sMICA) and sULBP2 levels to the patient disease course, we found only sULBP2, in contrast to sMICA, to be a strong predictor of poor prognosis even in early disease stage. On the one hand, the association between high sULBP2 and shortened overall survival might indeed reflect an immune escape of the tumor, associated with an accelerated disease progression. On the other hand, one cannot exclude that elevated sULBP2 levels could originate from an exceptionally strong ligand expression in a highly progressive dedifferentiated tumor phenotype. If the latter is the case, then mechanisms that drive ULBP2 expression in such tumors await further investigation.

References

1. Champsaur M, Lanier LL. Effect of NKG2D ligand expression on host immune responses. Immunol Rev 2010; 235:267-85; PMID:20536569
2. Gasser S, Orsulic S, Brown EJ, Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 2005; 436: 1186-90; PMID:15995699; http://dx.doi.org/10.1038/nature03884
3. Heinemann A, Zhao F, Pechlivanis S, Eberle J, Steinle A, Diederichs S, et al. Tumor suppressive microRNAs miR-34a/c control cancer cell expression of ULBP2, a stress- induced ligand of the natural killer cell receptor NKG2D. Cancer Res 2012; 72:460-71; PMID: 22102694; http://dx.doi.org/10.1158/0008-5472.CAN-11-1977
4. Hermeking H. The miR-34 family in cancer and apoptosis. Cell Death Differ 2010; 17:193-9; PMID: 19461653; http://dx.doi.org/10.1038/cdd.2009.56
5. Agostini M, Tucci P, Killick R, Candi E, Sayan BS, Rivetti di Val Cervo P, et al. Neuronal differentiation by TAp73 is mediated by microRNA-34a regulation of synaptic protein targets. Proc Natl Acad Sci U S A 2011; 108:21093-8; PMID:22160687; http://dx.doi.org/10.1073/pnas.1112061109
6. Rinaldo C, Prodosmo A, Siepi F, Moncada A, Sacchi A, Selivanova G, et al. HIPK2 regulation by MDM2 determines tumor cell response to the p53-reactivating drugs nutlin-3 and RITA. Cancer Res 2009; 69:6241-8; PMID:19638586; http://dx.doi.org/10.1158/0008-5472.CAN-09-0337
7. Textor S, Fiegler N, Arnold A, Porgador A, Hofmann TG, Cerwenka A. Human NK cells are alerted to induction of p53 in cancer cells by upregulation of the NKG2D ligands ULBP1 and ULBP2. Cancer Res 2011; 71:5998-6009; PMID:21764762; http://dx.doi.org/10.1158/0008-5472.CAN-10-3211
8. Li H, Lakshmikanth T, Garofalo C, Enge M, Spinnler C, Anichini A, et al. Pharmacological activation of p53 triggers anticancer innate immune response through induction of ULBP2. Cell Cycle 2011; 10:3346-58; PMID:21941086; http://dx.doi.org/10.4161/cc.10.19.17630
9. Paschen A, Sucker A, Hill B, Moll I, Zapatka M, Nguyen XD, et al. Differential clinical significance of individual NKG2D ligands in melanoma: soluble ULBP2 as an indicator of poor prognosis superior to S100B. Clin Cancer Res 2009; 15:5208-15; PMID:19671853; http://dx.doi.org/10.1158/1078-0432.CCR-09-0886
10. Schwinn N, Vokhminova D, Sucker A, Textor S, Striegel S, Moll I, et al. Interferon-gamma down-regulates NKG2D ligand expression and impairs the NKG2D-mediated cytolysis of MHC class I-deficient melanoma by natural killer cells. Int J Cancer 2009; 124:1594-604; PMID:19089914; http://dx.doi.org/10.1002/ijc.24098