Metastasis suppressor function of TRAIL-R in mice- implications for TRAIL-based therapy in humans?

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Abstract

TRAIL is a promising candidate for cancer therapy, as it can induce apoptosis specifically in tumor cells but not in normal cells. While earlier mouse tumor studies revealed a strong tissue dependency of TRAIL and its death receptor in suppressing primary tumorigenesis or experimental metastases, we recently found that TRAIL-R inhibits lymph node metastases without affecting primary tumor formation in a mouse model of multistage skin tumorigenesis. This finding uncouples the role of TRAIL in primary tumorigenesis from metastasis formation, likely by sensitization of previously TRAIL-resistant tumor cells upon detachment, an early step required for metastasis formation. Therefore, TRAIL-R is a novel metastasis suppressor, suggesting that TRAIL-related tumor therapy might be most effective in primary tumors and early metastatic cancers, before selection for TRAIL resistance occurs.

The apoptosis-inducing TRAIL system in primary tumorigenesis and metastasis

The TNF-related apoptosis inducing ligand (TRAIL/Apo2L) is a transmembrane protein expressed on activated cells of the immune system such as NK cells, T and B lymphocytes, monocytes, and dendritic cells. TRAIL binds to receptors containing a death domain, called death receptors. In humans, TRAIL has two receptors containing a death domain, TRAIL-R1 (DR4) and TRAIL-R2 (DR5/KILLER/TRICK2/APO2), while there is only one death receptor in mice equally homologous to both human death receptors, called TRAIL-R (1). In addition, in both humans and mice TRAIL can also bind to three different receptors with no functional death domains, also referred to as “decoy” or “regulatory receptors”. However, the physiological function of these receptors with respect to TRAIL-signaling has not yet been fully investigated (1). Ligation of TRAIL death receptors can activate the extrinsic apoptosis pathway by recruiting the adaptor molecule FADD resulting in Caspase-8 and Caspase-3 activation (2). For the biopharmaceutical industry TRAIL has raised considerable interest, as TRAIL was shown to preferentially kill tumor cells while non-transformed cells remained TRAIL-resistant *in vitro* and *in vivo.* Also, at least one of the two TRAIL death receptors is expressed on all cancer cells examined. Therefore, targeting the apoptosis-inducing TRAIL system is a promising anti-tumor strategy with little side effects. Recombinant TRAIL or agonistic antibodies against the human TRAIL death receptors are currently in clinical trials. Although 30-50% of tested human cancer cell lines are TRAIL-resistant (3), most can be sensitized to TRAIL

by prior treatment with chemotherapeutics, proteasome inhibitors or γ-irradiation (1).

Due to these tumor cell-specific effects, it was suggested that a physiological role of the TRAIL system is tumor suppression. Indeed, transplantation of TRAIL-sensitive tumor cells into syngeneic TRAIL-deficient mice led to increased subcutaneous tumor growth and experimental liver metastases (4, 5). This observed tumor surveillance effect of TRAIL was at least in part dependent on expression of TRAIL by activated liver NK cells (4, 6). Interestingly, lung NK cells do not express TRAIL (6), which could explain why experimental lung metastases were not accelerated in TRAIL-deficient mice (5, 7) and demonstrates a tissue-specific effect of TRAIL-mediated tumor immune surveillance (6).

Autochthonous mouse tumor models using TRAIL and TRAIL-R-deficient mice also revealed a strong tissue dependency of tumor suppression by TRAIL and TRAIL-R. In some tissues, such as liver, lymphoid tissues (8), and muscle, the TRAIL system can inhibit primary tumor formation, while in epithelial tissues such as mammary gland and colon it had no observable effect (1). Consistently, we found recently that neither carcinogen-induced skin tumor initiation nor tumor growth or conversion was accelerated in TRAIL-R-deficient mice (9). So far, the reason for this observed tissue dependency of the tumor suppressor function of the TRAIL system is unclear. Although both mammary and skin cancer cell lines express TRAIL-R on their surface (9, 10), it remains to be determined whether primary tumor cells are TRAIL-resistant *in vivo* and/or whether insufficient TRAIL-

expressing killer cells of the immune system gain access to those primary epithelial tumors. In addition, the tumor suppressor function of the TRAIL system in some tissues could be dependent on non-apoptotic functions of TRAIL and TRAIL-R signaling; there is some evidence that TRAIL-R can have an anti-inflammatory effect resulting in tumor protection (8).

TRAIL-R as a metastasis suppressor

Despite no difference in primary skin tumorigenesis in TRAIL-R-deficient mice, they developed a greater number of metastases to the lymph nodes than wild-type littermates (9). This was the first demonstration that TRAIL-R signaling could inhibit metastasis from autochthonous tumors, and that TRAIL-R can exert its anti-tumor effect in a tumor stage-dependent manner. Interestingly, carcinoma cells isolated from primary skin tumors of wild-type mice were initially TRAIL resistant. Since the latency of primary skin tumors was not affected by TRAIL-R deficiency, we concluded that selection for TRAIL resistance of skin carcinoma cells during primary tumorigenesis was unlikely. An early required step in metastasis formation is detachment of tumor cells from the extracellular matrix. In epithelial cells this can lead to anoikis as well as to sensitization of cells to apoptosis (11). While anoikis appeared to be TRAIL-R-independent, detachment increased sensitivity of skin carcinoma cells to exogenously added TRAIL (9). Detachment-induced TRAIL sensitization has also been reported in breast carcinoma cells (12) and ovarian carcinoma cells (13). The sensitization of skin carcinoma cells to TRAIL by detachment correlated with downregulation of ERK activity and inhibition of ERK activity sensitized attached carcinoma cells to

TRAIL (9). In summary, the sensitization of carcinoma cells to TRAIL by detachment, provides an explanation for how the TRAIL system can suppress metastases without affecting primary tumor formation. Furthermore, our data suggest that even if the primary tumor is TRAIL resistant there could be a natural window of TRAIL sensitivity during early metastasis formation.

In another tumor study we found that tumor free survival as well as the tumor spectrum of irradiated TRAIL-R-deficient mice was not significantly different from wild-type mice, with the majority of observed tumors in both genotypes being disseminated lymphomas of B cell origin. Interestingly, in TRAIL-R-deficient mice with lymphoma we observed increased dissemination of lymphoma to Peyer's patches, mesenteric lymph nodes, and pancreas, although primary lymphomagenesis was not affected (manuscript in preparation). These data confirmed our finding of a specific antimetastatic effect of TRAIL-R expression in mice. It will be interesting to further analyze whether the anti-metastatic effect of endogenous TRAIL signaling via TRAIL-R is particularly important for lymph node metastases. This could be facilitated through increased TRAIL expression in lymphatic cells or increased sensitization of tumor cells to TRAIL in the lymphatic system, for example through cytokines such as γIFN (14).

Genes that reduce the metastatic propensity of tumor cells without affecting their tumorigenicity are defined as metastasis suppressors (15). By these criteria, *Trail-r* is a metastasis suppressor gene. Death of cancer patients is mostly due to metastatic spread of the primary tumor. Therefore, it is necessary to improve treatment of metastatic disease, for example by increasing expression and

function of metastasis suppressors. To date, more than twenty metastasis suppressors have been identified, many of which encode for intracellular signaling molecules (15) making them difficult to target therapeutically. By contrast, TRAIL receptors are expressed on the cell surface, and several TRAIL receptor agonists are already in clinical trials (1).

Implications of the specific role of TRAIL-R in metastasis suppression for tumor therapy

Increased selective pressure against tumor suppressors leads to their loss of function during primary tumorigenesis. For example the tumor suppressor p53 is mutated in more than 50% of all primary human cancers. Similarly, most metastasis suppressors are lost, mutated, or downregulated during the metastatic process. If TRAIL-R is also a metastasis suppressor in humans, we would predict loss of TRAIL-R-mediated apoptosis signaling in metastatic tumors when compared to primary tumors. Indeed, there is evidence for selective pressure on inactivating the TRAIL apoptosis pathway specifically in metastases by mutation or downregulation of the TRAIL death receptors. This has been observed for example in metastases of breast cancer and melanoma (16, 17). Furthermore, metastatic cell lines also showed greater TRAIL resistance than their counterparts isolated from primary tumors (18). Interestingly, Caspase-8, an important downstream signaling component of death-receptors has also been identified as a metastasis suppressor (19). The metastasis suppressor function of caspase-8 could be also due to its function as a downstream mediator of death receptor-mediated apoptosis.

Although both Caspase-8 as well as TRAIL-R have been described as metastasis suppressors, depending on the context both can also suppress primary tumor development. A tumor suppressor function of the TRAIL apoptosis pathway could result in TRAIL-resistance of the primary tumor. In contrast, a metastasis suppressor role of the TRAIL system could lead to TRAIL-resistance at the metastasis stage while the primary tumor might remain sensitive or at least sensitizable to TRAIL-receptor-targeting drugs. In the future, it will be important to define whether selection for TRAIL-resistance happens by irreversible gene mutation of TRAIL death receptors or of other essential signaling components of the apoptotic machinery, or by reversible regulation of expression of apoptotic modulators. Fortunately, TRAIL resistance is often due to reversible expression of TRAIL-R pathway modulators such as upregulation of anti-apoptotic molecules such as cFLIP, anti-apoptotic Bcl family proteins, and IAPs, or by downregulation of proapoptotic molecules (2). Therefore, TRAIL-sensitivity can be easily increased by additional drugs such as the proteasome inhibitor Bortezomib, or conventional chemotherapeutics such as cisplatinum or paclitaxel, all of which are currently being tested in clinical trials together with TRAIL-receptor targeting drugs (1).

In conclusion, we recently found evidence for a metastasis suppressor function of the apoptosis-inducing TRAIL-R in mice, and current evidence suggests a similar function in humans. More studies are needed to determine the mechanism(s) and conditions by which tumor cells can acquire resistance to TRAIL during

metastasis formation. These studies are likely to have major impact for TRAIL-receptor based cancer therapy.

Figure legend

A model for inhibition of metastasis by the apoptosis-inducing TRAIL system. Primary skin carcinomas are initially resistant to TRAIL (R), but become sensitized (S) by detachment. Activated NK cells expressing TRAIL are the likely effector cells that kill detached carcinoma cells via TRAIL-R-mediated apoptosis. Detached carcinoma cells not expressing functional TRAIL-R are resistant to TRAIL induced apoptosis, which enhances their metastatic ability. The TRAIL system could generate selection pressure during metastasis to develop TRAIL resistance, either through mutations or downregulation of TRAIL-R or other components of the TRAIL apoptosis pathway, which could have a negative impact towards TRAIL-based therapy of established metastases.

Acknowledgement:

This work was supported by NIH research grants R01 CA070414 and R01 CA099517 to C.J.K.

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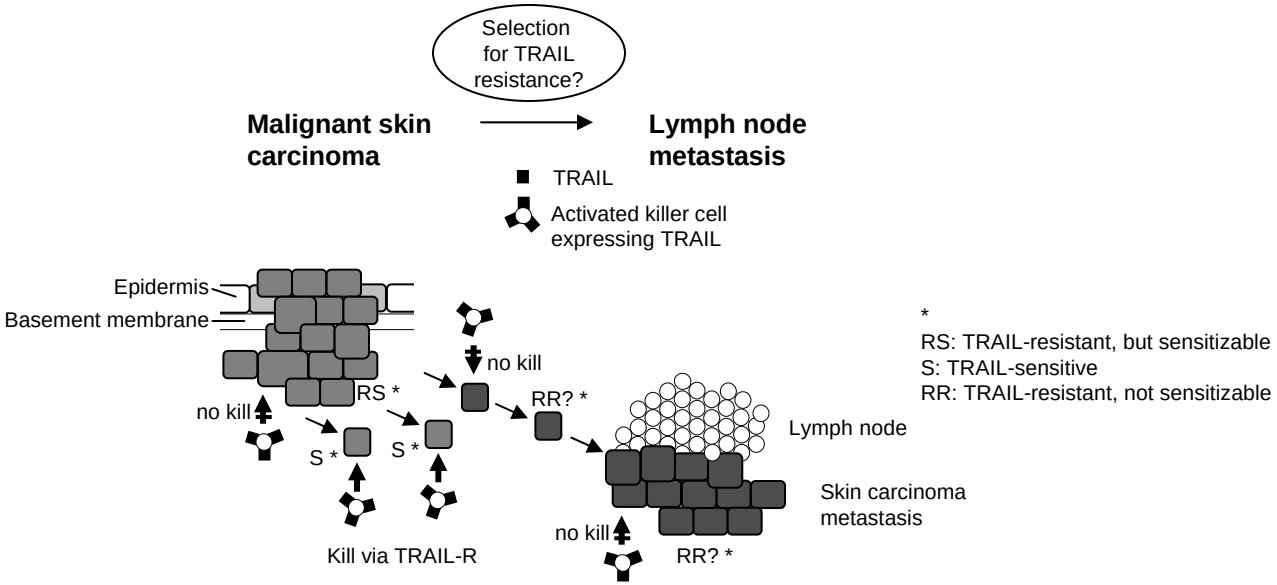

TRAIL-R expression inhibits metastasis formation of skin carcinomas by mediating TRAIL-induced apoptosis induced in detached skin carcinoma cells. Cell lines from primary skin carcinomas are resistant to TRAIL (RS), but detachment of WT skin carcinoma cells leads to sensitization of those cells to TRAIL by detachment (S). Activated NK cells express TRAIL, have been shown to mediate anti-tumor effects of TRAIL, and are likely to be the effector cells killing detached skin carcinoma cells via TRAIL-R-mediated apoptosis. This could potentially lead to selection pressure resulting in TRAIL resistance (RR) in detached, early metastasizing cells, either through irreversible mutations or downregulation of essential signaling components of the apoptosis-inducing TRAIL/TRAIL-R pathway.