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Targeted cancer immunotherapy
Mimicking physiological trans-presentation of IL-15

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Under physiological conditions, the trans-presentation of interleukin-15 (IL-15) by the IL-15 receptor α on the cell surface allows to confine and tune the IL-15-mediated immune responses. Therefore, targeting strategies that mimic this situation at the tumor sites appear especially promising for anticancer immunotherapy.

Interleukin (IL)-15 belongs to the common receptor γ-chain cytokine family, which also includes IL-2, IL-4, IL-7, IL-9 and IL-21. IL-15 plays an important role in the development, activity and persistence of natural killer (NK) cells and CD8$^+$ T cells, showing great potential to promote antitumor immune responses. Thus, next to IL-2, which has been approved for treatment of metastatic renal cell carcinoma and metastatic melanoma, IL-15 is attracting increasing attention, ranking on the top of the National Cancer Institute's list of agents considered to have high potential for cancer immunotherapy.[1] Although IL-15 shares many functions with IL-2, like the ability to stimulate the proliferation of NK and T cells, the generation of cytotoxic T cells and the activation of NK cells, it also has properties different from that of IL-2. Thus, unlike IL-2, IL-15 seems not to be involved in the activation-induced cell death (AICD) of effector CD8$^+$ T cells and in the maintenance of regulatory T cells. Furthermore, preclinical studies suggest that IL-15 might be less toxic than IL-2. Hence, benefits in terms of efficacy and safety are expected, and several clinical trials involving IL-15 are currently being conducted.[2]

IL-2 and IL-15 share the β and common γ chain of their receptors (IL-2/15Rβγ$_c$), but specifically bind to their respective α receptor chains (IL-2Rα/IL-15Rα). IL-15 binds intracellularly with high affinity to IL-15Rα, forming a complex that shuttles from the endoplasmic reticulum to the cell surface, where IL-15Rα presents IL-15 mainly in trans to IL-2/15Rβγ$_c$ expressing neighbor cells, although cis-presentation is also possible. Recycling of the whole complex between the cell surface and endosomes has been described. Thus, under physiological conditions, a confined, long-lasting membrane presentation of IL-15 can be achieved that favors a highly regulated system.[3]

Although IL-2/15Rβγ$_c$ can be stimulated by IL-15 alone, it was shown in mice that a soluble complex of IL-15 and IL-15Rα-Fc is much more effective, leading to stronger proliferation of memory CD8$^+$ T cells and NK cells in vitro and in vivo. Also antitumor activity was increased, reducing tumor burden and increasing the survival of B16 melanoma-bearing mice.[4,5] Similar results have been reported on a fusion protein composed of IL-15 and the fragment of the IL-15Rα chain involved in ligand binding (extended sushi domain). In comparison with IL-15 alone, treatment with the fusion protein was more efficient in mouse lung or liver metastatic melanoma (B16F10 cells) models as well in a human colon carcinoma xenograft (HCT 116 cells) model, as demonstrated by reduced metastasis formation, tumor growth inhibition and increased survival.[6] Thus, the presentation of IL-15 in the context of the IL-15Rα chain seems to clearly improve the antitumor potential of this cytokine.

The relevance of localized IL-15 presentation at the tumor site for anticancer immune responses was shown in a *Rag1*$^{-/-}$ mouse model bearing IL-15-secreting tumor cells. Large tumors grew in the presence of neutralizing IL-15 antibodies, but were eradicated by an NK cell-mediated immune response upon antibody withdrawal. In this setting, the co-expression of IL-15Rα by tumor cells was required for the efficient induction of densely granulated NK effector cells in the tumor microenvironment. The regression of IL-15-secreting tumors did not stop the growth of contralateral non-IL-15-secreting control tumors, suggesting that the effect of IL-15 was restricted to the local microenvironment.[7] In a pancreatic tumor RIP1-Tag2 mouse model, regression of established solid tumors after IL-15/IL-15Rα-Fc complex treatment could be ascribed to the stimulation of endogenous, tumor-resident CD8$^+$ T cells.[8] In addition, targeted delivery of IL-15 as an EDB-directed antibody-IL-15 fusion protein resulted in the accumulation of the fusion protein in the tumor and consecutive growth inhibition in a syngeneic F9 teratocarcinoma mouse model.[9] Thus, the localization of IL-15 at the tumor site constitutes an important factor for the generation of efficient antitumor immune responses.

Recently, by generating a fusion protein composed of a tumor-directed antibody, IL-15 and a IL-15Rα chain domain, we

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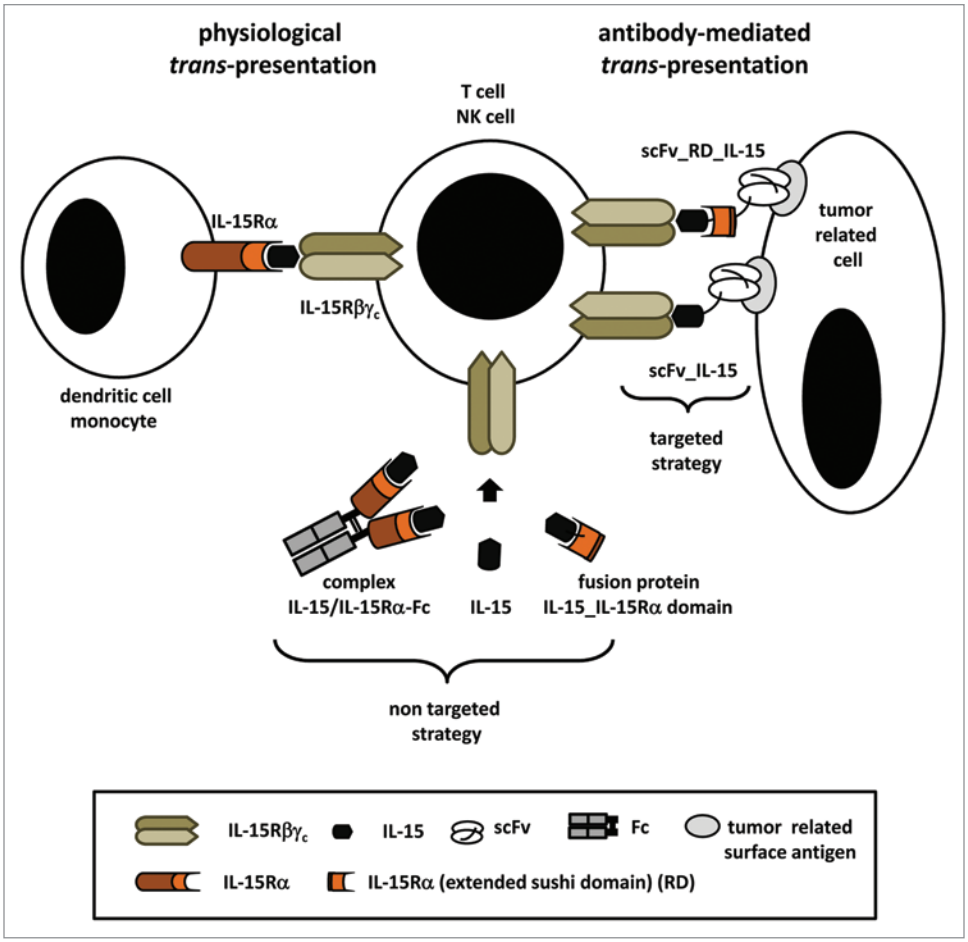

Figure 1. Schematic illustration of interleukin-15 trans-presentation. Under physiological conditions interleukin-15 (IL-15) binds to the IL-15Rα chain and is presented on the cell surface of dendritic cells and monocytes in trans to IL-15Rβγ$_c$ expressing T or NK cells. Trans-presentation can be simulated in a non-targeted form in solution by complexing or fusing IL-15 to a ligand binding fragment of the IL-15Rα chain. Alternatively, trans-presentation can be achieved in a targeted manner by fusing IL-15 alone or in combination with the interacting IL-15Rα domain to a tumor-directed antibody moiety. Thus, antibody-mediated binding of the fusion protein mimics cell surface presentation of IL-15 at the tumor site. scFv, single-chain Fv.

have combined both aspects: presentation of IL-15 in the IL-15Rα context and targeting IL-15 presentation to tumor cells, mimicking the physiologic trans-presentation of IL-15 at the tumor site (**Fig. 1**).[10] We could show in vitro, that targeted presentation of IL-15 linked to the extended sushi domain of IL-15Rα (RD) was more effective in stimulating T cells in terms of proliferation and cytotoxicity than the targeted presentation of IL-15 alone. In addition, we demonstrated in a metastatic B16-transfectant tumor mouse model that optimal antitumor effects were achieved by the antibody-RD-IL-15 fusion protein, while both antibody-IL-15 or non-targeted RD-IL-15 were less effective. Thus, this particular strategy of mimicking physiological trans-presentation of IL-15 at the tumor site seems to be a promising approach for an improved application of IL-15 in cancer immunotherapy.

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