TNF receptor superfamily-based targeted cancer immunotherapy

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Chapter 7

Summary
The Tumor Necrosis Factor ligand superfamily (TNFSF) comprises at least 19 ligands that serve as key mediators of immune regulation and inflammation and as inducers of apoptosis. Ligands of the TNFSF bind and interact with specific receptors of the TNF Receptor superfamily (TNFRSF), a group of homologous transmembrane proteins, some of which bear an intracellular “death domain” (e.g. TNFR, Fas, TRAIL-R1&2) and are able to directly mediate apoptosis. However, 'proinflammatory' TNFRSF members (e.g. CD40) may also enhance apoptotic responses, whereas death receptors may trigger proinflammatory pathways.

The research presented in this thesis focuses on the preclinical development of novel cancer immunotherapy approaches by exploiting the unique tumor-selective pro-apoptotic and potent immune stimulatory features of various ligands of the TNFSF. In this respect, TRAIL is of particular interest as it selectively induces apoptosis in tumor cells with minimal or no toxicity in normal cells and tissues. Therefore, inducing apoptosis by soluble TRAIL or agonistic antibodies which target DR4 or DR5 were considered to be promising approaches in cancer therapy. Consequently, TRAIL-R agonists have been extensively evaluated in clinical trials, alone or in combination with various anticancer drugs. However, in general the majority of clinical trials demonstrated that TRAIL receptor agonists (both soluble-TRAIL formulations and agonistic anti-TRAIL-R antibodies) were well-tolerated but exerted only limited efficacy in cancer patients.

Therefore, in Chapter 3, we developed a novel bispecific antibody-based approach that promotes melanoma-directed pro-apoptotic activation of DR5. To this end, a recombinant bispecific antibody, designated MCSPxDR5, was constructed with both high binding affinity for the melanoma-associated antigen MCSP and potent agonistic activity towards DR5. MCSPxDR5 showed potent MCSP-directed pro-apoptotic activity towards MCSP-positive melanoma cells with essentially no or minimal toxicity towards normal cells. The antitumor activity by MCSPxDR5 was enhanced by cross-linking of its IgG domain by either by artificial crosslinker or Fc receptors on myeloid immune effector cells.

Importantly, MCSPxDR5 induced potent apoptosis in 11 out of 11 MCSP-expressing primary patient-derived melanoma cells, up to 70%. In conclusion, we presented for the first time a bispecific antibody-based approach that promotes melanoma-directed pro-apoptotic activation of DR5. This novel approach may be of value for the targeted treatment of melanoma and other MCSP-expressing malignancies.

Tumor immune surveillance concept states that the immune system continuously strives to identify cancerous and/or precancerous cells and eliminate
them before they can develop into uncontrollable disease. Nevertheless, despite tumor immune surveillance, cancer cells do develop in the presence of an apparently fully functional immune system. More recently, the concept of tumor immunoediting was conceived which is composed of 3 phases: elimination (largely analogous to cancer immune surveillance); equilibrium, an extended phase of tumor dormancy in which tumor cells and the immune system are in dynamic equilibrium that keeps tumor cell expansion in check; and finally escape, in which tumor cells emerge with reduced immunogenicity or with powerful immunosuppressive mechanisms that are able to inhibit antitumor immune responses. The latter phase leads to progressively growing tumors and finally full-blown malignant disease.

Recent data demonstrated that tumors can evade recognition by the immune system through cell surface upregulation of phagocyte-inhibitory signals. CD47 is a prominent “don’t eat me” signal that binds to signal-regulatory protein alpha (SIRPα) expressed on phagocytes. Cancer cells often mis-use this ‘don’t eat me’ mechanism by overexpressing CD47, which allows them to evade phagocytic clearance and subsequent immunogenic processing.

In Chapter 4, we explored the possibility to counteract CD47-mediated ‘don’t eat me’ signaling by cancer cells, while simultaneously induce apoptosis in the same cells by exploiting a novel fusion protein in which a CD47-blocking antibody fragment (scFvCD47) is genetically fuse to TRAIL. This new fusion protein, designated anti-CD47:TRAIL, was designed to 1) block CD47-SIRPα interaction and hereby potentiate phagocytosis induced by therapeutic anticancer antibodies, and 2) concurrently trigger CD47-restricted apoptotic cell death in malignant cells. Our data show that indeed fusion protein anti-CD47:TRAIL triggered apoptosis in CD47pos B-cell lines and in 4 out of 5 patient-derived primary B-NHL samples, up to 90%. Furthermore, the results showed that anti-CD47:TRAIL selectively enhanced antibody-mediated phagocytosis of B-NHL cells by rituximab in a target antigen-restricted manner. In conclusion, anti-CD47:TRAIL effectively blocks CD47-mediated “don’t eat me” signaling, promotes RTX-induced phagocytosis by granulocytes and triggers CD47-restricted apoptosis in malignant B cells.

According to immune editing concept in the escape phase tumor cells can emerge that have developed powerful immunosuppressive mechanisms which allow them to inhibit antitumor immune responses. In particular, Programmed death 1 (PD-1) and its ligand PD-L1 play a key role in this tumor immune escape and
the formation of an immune suppressive tumor microenvironment. The immune inhibitory activity of the PD1/PD-L1 checkpoint is important for maintaining self-tolerance and crucial in the protection against tissue damage when the immune system is activated in response to infection. However, under immune attack, cancer cells overexpress PD-L1 which interacts with PD-1 receptor on anticancer T cells, thereby suppressing their activity and inducing immune escape. Antibodies that block PD-L1/PD-1 immune checkpoints can restore the activity of such functionally-impaired antitumor T cells. However, only a subset of cancer patients responds to current PD-L1/PD-1-blocking strategies, highlighting the need for further advancements in PD-L1/PD-1-based immunotherapy.

In Chapter 5, we report on a novel approach designed to combine PD-L1 checkpoint inhibition with the tumor-selective induction of apoptosis by TRAIL. In brief, a new bi-functional fusion protein, designated anti-PD-L1:TRAIL, was constructed comprising a PD-L1-blocking antibody fragment genetically fused to the extracellular domain of the pro-apoptotic tumoricidal protein TRAIL. Fusion protein anti-PD-L1:TRAIL exhibited four major biological functions: 1) induction of TRAIL-mediated cancer cell death after binding to tumor-expressed PD-L1, 2) reactivation of antitumor T-cells by blocking of PD-L1/PD-1 interaction, 3) converting suppressive monocytes/macrophages/DCs into pro-apoptotic effector cells that trigger TRAIL-mediated cancer cell death and 4) enhancement of IFN-γ production by immune effector cells. Moreover, in an in vitro model system, treatment with anti-PD-L1:TRAIL enhanced the activity of antigen-experienced anti-CMV pp65 T-cells by significantly enhancing their capacity to secrete IFN-γ. In conclusion, combining PD-L1 checkpoint inhibition with TRAIL-mediated induction of apoptosis using anti-PD-L1:TRAIL yields promising multi-fold and mutually reinforcing anticancer activity that may be exploited to enhance the efficacy of therapeutic PD-L1/PD-1 checkpoint inhibition.

As described in Chapter 2, most ‘first-generation’ TNFRSF agonists do not effectively activate their corresponding cognate receptor and reversely ubiquitous TNFRSF receptor cross-linking by agonistic antibodies may trigger severe toxicity as is evidenced by dose-limiting liver toxicity of CD40 and Fas agonistic antibodies.

In Chapter 6, we aimed to address these issues by developing a novel pretargeting strategy using recombinant fusion proteins in which a soluble form of TRAIL, FasL or CD40L is genetically fused to a high-affinity anti-fluorescein scFv antibody fragment (scFvFITC). Fusion proteins scFvFITC:sTRAIL and scF-
vFITC:sFasL induced potent target antigen-restricted apoptosis in a panel of different cancer lines and primary patient-derived cancer cells only when pretargeted with a relevant FITC-labeled antitumor antibody. Of note, scFvFITC:sTRAIL or scFvFITC:sFasL synergized with velcade-induced apoptosis in 3 out of 3 primary patient-derived ovarian cancer cells. In a similar pretargeting setting, fusion protein scFvFITC:CD40L promoted tumor-directed maturation of immature monocyte-derived dendritic cells (iDCs). Importantly, the activity of these scFvFITC:TNFSF fusion proteins can be inhibited in the presence of a non-toxic fluorescein, allowing the possibility to titrate their activity and/or attenuate on target/off tumor toxicity.