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Fractalkine gene therapy for neuroblastoma is more effective in combination with targeted IL-2

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Abstract

The induction of tumor protective immunity against neuroblastoma remains a major challenge for active immunotherapy. Fractalkine is a unique Th1 CX3C chemokine known to induce adhesion and migration of leukocytes mediated by both, a membrane-bound and soluble form, respectively. Here, we tested the hypothesis that chemokine gene therapy with fractalkine (FKN) induces an effective anti-neuroblastoma immune response amplified by targeted IL-2 using the anti-GD2 antibody ch14.18 fused with IL-2 (ch14.18-IL-2).

For this purpose, NXS2 cells were genetically engineered to stably produce murine FKN (NXS2-FKN). Transcription and expression of the mFKN gene in tumor tissue of mice inoculated with NXS2-FKN cells were demonstrated in vivo. Importantly, mFKN exhibited a reduction in primary tumor growth and spontaneous liver metastases in syngenic A/J mice. This effect was boosted by targeted IL-2 using small non-curative doses of ch14-18-IL-2. The amplification of the FKN induced immune response was specific, since a non-specific antibody-IL-2 fusion protein ch225-IL-2 was ineffective.

In summary, we demonstrated for the first time that chemokine gene therapy is amplified by targeted IL-2 suggesting a combination of both strategies as an adjuvant therapy for neuroblastoma.

Keywords: Fractalkine; GD2; Ch14.18-IL-2; Neuroblastoma; Gene therapy; Immunotherapy

1. Introduction

The phenomenon of tumor-associated leukocytes is considered to be residual evidence of the host's ineffective antitumor immune response. Therefore, a major goal of immunotherapy is further accumulation of such immune effector cells in the tumor microenvironment. Based on these considerations, chemokines attracted the attention of tumor immunologists,

Abbreviations: mFKN, murine fractalkine; IL, interleukin; GD_2, disialoganglioside GD_2; mAb, monoclonal antibody; NB, neuroblastoma; s.c., subcutaneous; i.v., intravenous; NK, natural killer.

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since they are very potent chemotactic agents. Chemokines can be divided into four major families based on the protein structure: CC, CXC, C and CX3C depending on the configuration of cysteine residues near the NH_2-terminus. Furthermore, chemokines are characterized by function. They are involved in lymphocyte homeostasis and inflammation. Homeostatic chemokines, such as SLC (secondary lymphoid tissue chemokine), are constitutively produced in lymphoid tissue involved in maintaining physiological migration of cells that mainly belong to the adoptive immune system. In contrast, inflammatory chemokines, such as IP10 (interferon-inducible protein 10), are expressed upon stimulation by pro-inflammatory cytokines or pathogenic agents and specialized for the recruitment of immune effector cells [1].

Fractalkine (FKN), also referred as neurotactin or CX3CL1, is an unusual chemokine in terms of its structure. It is a member of the chemokine subfamily characterized by the CX3C motif with three amino acids between the two terminal cysteines. The most important difference between FKN and other chemokines is the fact that it consists of a 241 amino acid long mucin-like stalk extending the chemokine domain away from the cell surface [2,3]. To date, FKN and the newly described CXCL16 are the only chemokines identified that share this kind of structure [4]. As a result of this unique structure, FKN is bifunctional. The soluble form of FKN, comprising the chemokine domain and most of the stalk region, is produced by proteolytic cleavage at a membrane-proximal dibasic cleavage site, which acts like a classic chemokine attracting leukocytes through a gradient. In contrast, the cell-surface bound FKN promotes strong adhesion of leukocytes to the FKN producing cell without requiring additional adhesion molecules such as selectins or integrins [5]. FKN is expressed predominantly by endothelial cells and its expression is inducible upon stimulation with TPA (12-*o*-tetradecanoylphorbol-13-acetate), LPS, and Th1 cytokines, such as IFN-γ TNF-α or IL-1, but not Th2 type cytokines, such as IL-4 or IL-13, which even suppress this induction [6]. FKN exerts its dual function through its receptor CX3CR1, previously known as V28. This receptor is a seven-transmembrane protein that contains several motifs conserved among the chemokine receptor superfamily and mainly expressed on monocytes, $CD56^+/CD16^+$ NK cells and $CD8^+$ T cells [7]. Moreover, the membrane-bound form of FKN can induce INF-γ production by cells so that a paracrine feedback loop may exist between FKN and INF-γ [8]. In this regard, FKN plays an important role in a Th1 type immune response as amplification circuit of polarized Th1 cell. Based on these unique characteristics of FKN, we selected this chemokine for gene therapy of neuroblastoma in combination with targeted IL-2.

The concept of targeted IL-2 has been described elsewhere [9]. Briefly, human/mouse chimeric anti-GD2 antibody ch14.18 was genetically fused to the coding sequence of human IL-2 to generate the ch14.18-IL-2 fusion protein combining the unique GD2 targeting ability of ch14.18 with the functional activity of IL-2. It was demonstrated that directing IL-2 into the tumor microenvironment amplifies a suboptimal cancer vaccine [10]. Therefore, we tested the hypothesis that T-cells attracted to the neuroblastoma microenvironment by FKN gene therapy are activated by targeted IL-2 leading to an optimized anti-neuroblastoma immune response.

2. Materials and methods

2.1. Construction of a plasmid encoding for mFKN and generation of a stable NXS2 cell clone expressing high levels of mFKN

The full-length cDNA sequence encoding for murine fractalkine (mFKN) was cloned from total RNA of murine breast cancer cells D2F2 using primers 5′-NheI GCTAGCATGGCTCCCTCGCC GCTCGCG-3′ (sense) and 3′-EcoRI GAATTCTCA CACTGGCACCAGGACGTA-5′ (antisense) by RT-PCR. The sequence was verified by molecular sequencing. Then, the fragment was subcloned from pCR2.1 into a mammalian expression vector pIRES with restriction enzyme NheI and EcoRI (pIRES-FK). NXS2 mouse neuroblastoma cells were grown in DMEM (PAA Laboratories, Germany) with high glucose supplement with 10% FCS (PAA laboratories, Germany) and 100 μg/ml penicillin–streptomycin (Invitrogen, USA). pIRES-FKN was transfected into NXS2 with superfect (Qiagen,