TGF-BETA VACCINE

Field of the Invention

The present invention relates to novel polypeptides, which are derived from transforming growth factor beta 1 (TGFβ1; TGFb1) as well as polynucleotides encoding such polypeptides and compositions comprising such peptides. The invention also concerns uses, and methods of using, said polypeptides, polynucleotides, and compositions.

Background of the Invention

TGFb is a multifunctional cytokine with a key role in the regulation of the immune system. There are four isoforms, of which isoform1 (TGFb1) is particularly important in T-cell immunity. In the context of cancer, TGFb1 disarms various immune cells like cytotoxic T-cells (CTLs), tumor-associated neutrophils and Natural Killer (NK) cells. It also contributes to tumor vascularization and metastasis. Consequently, TGFb1 is a key inhibitory molecule in the tumor microenvironment (TME), contributing to a down-regulation of the immune system's anti-tumor machinery and enabling immune-evasion by cancer cells.

In recent studies of murine models of metastatic liver cancer, TGFb1 has also been seen to contribute to a decrease in the efficiency as cancer therapies of Immune Checkpoint Blockers (ICBs), such as PD-L1 blockade.

Summary ~~of the Invention~~

The polypeptides of the present ~~invention~~disclosure are expected to be particularly effective at stimulating a beneficial immune response against TGFb1-expressing cells. The development of novel immune therapies for cancer requires a thorough understanding of the molecules that are involved in the pathogenesis as well as the specific proteins recognized by the immune system. In the clinical setting the induction of TGFb1-specific immune responses may directly kill TGFb1-expressing cancer cells, but more significantly it will support anti-cancer immune responses in general by suppressing the immune suppressive function of TGFb1. Targeting TGFb1 and TGFb1-expressing cells, e.g. by vaccination with the polypeptides of the present ~~invention~~disclosure, will consequently be highly synergistic with additional anti-cancer immunotherapy, such as Immune Checkpoint Blockers (ICBs).

TGFb1 is a dimeric cytokine which shares a cysteine knot structure connected together by intramolecular disulfide bonds. TGFb1 is synthesized as a monomeric 390-amino acid precursor protein, which is referred to interchangeably as: TGFb1 pre-protein; TGFb1 precursor; full-length TGFb1; pre-pro-TGFb1. The full-length sequence of the TGFb1 pre-protein is provided as SEQ ID NO: 1.

The TGFb1 pre-protein monomer has a molecular weight of about 25 kDa. The TGFb1 protein monomer has three distinct domains: the signal peptide (SP: amino acids 1-29; SEQ ID NO: 2), the latency associated peptide (LAP: amino acids 30-278; SEQ ID NO: 3) and the mature peptide (mature TGFb1: amino acids 279-390; SEQ ID NO: 4), as shown in Figure 1E.

The TGFb1 SP targets the protein to a secretory pathway; the SP is cleaved off in the rough endoplasmatic reticulum. TGFb1 monomers comprising the LAP and mature TGFb1 may dimerize in the endoplasmic reticulum via disulfide bridges between cysteine residues in the LAP (e.g. Cys 223 and Cys 225) and the mature TGFb1 peptide (e.g. Cys 356) to form a TGFb1 homodimer. This TGFb1 homodimer is referred to as the small latent complex (SLC). The SLC may be bound by so-called latent TGF-β-Binding Protein (LTBP) to form a larger complex referred to as the large latent complex (LLC). The LLC may be secreted into extracellular media (ECM). However, the presence of LAP and the LTBP prevent TGFb1 from binding to, and activating, its extracellular receptors. Active TGFb1 consists of a homodimer of mature TGFb1 peptides. There are various mechanisms by which the mature TGFb1 homodimer is released from LAP and LTBP, which include degradation of LAP by proteases, induction of conformational change in LAP by interaction with thrombospondin, and rupture of noncovalent bonds between LAP and TGFβ-1.

In one aspect, the present disclosure refers to a polypeptide which is an immunogenic fragment of human transforming growth factor 1 (TGFb1) and which comprises or consists of a sequence of at least 9 consecutive amino acids of SEQ ID NO: 1. In another aspect, the present disclosure refers to a polynucleotide encoding a polypeptide as disclosed herein, optionally comprised within a vector. In another aspect, the present disclosure refers to a composition comprising a polypeptide as disclosed herein, and optionally an adjuvant. In another aspect, the present disclosure refers to the polypeptide as disclosed herein, and/or the composition as disclosed herein for use in therapy.

In another aspect, the present disclosure refers to the use of the polypeptide as disclosed herein, and/or the composition as disclosed herein in the manufacture of a medicament for treating or preventing a disease or condition in a subject. In yet another aspect, the present disclosure refers to a method of stimulating TGFb1-specific T cells, the method comprising contacting the T cells with: the polypeptide as disclosed herein; and/or a composition as disclosed herein, wherein the composition comprises at least one polypeptide as defined herein.

An object of the present ~~invention~~disclosure is the development of a T-cell-mediated mechanism for depriving TGFb1 from TME. The present inventors investigated the existence of spontaneous TGFb1-specific T-cell responses *in vivo* by screening PBMCs from healthy donors and cancer patients. TGFb1-specific T-cell populations were then be isolated, expanded and characterized by various assays regarding HLA restriction, cytokines production and cytotoxicity.

The present inventors have identified regions of human TGFb1 which have greatest immunogenicity. Surprisingly, these immunogenic “hot spot” regions are located throughout the human TGFb1 pre-protein, including within the SP and LAP domains, as well as the mature TGFb1 peptide. The present inventors have also identified a sub-region within the

human TGFb1 LAP, i.e. positions 121-160 of SEQ ID NO: 1 (corresponding to the sequence of SEQ ID NO: 65), which harbours a greater frequency of immunogenic peptide sequences.

Thus, the present ~~invention~~disclosure provides a polypeptide which is an immunogenic fragment of human TGFb1 (SEQ ID NO: 1) and which comprises or consists of a sequence of at least 9 consecutive amino acids of SEQ ID NO: 1. The sequence of at least 9 consecutive amino acids of SEQ ID NO: 1 may correspond to a sequence of at least 9 consecutive amino acids of SEQ ID NOs: 2 or 65. The polypeptide may comprise or consists of up to 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 or 50 consecutive amino acids of SEQ ID NO: 1. The polypeptide may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 6, 42, 12, 23, 28, 49, 55, 63, 7-9, 43-45, 13-15, 24-26, 29-31, 50-52, 56-58, 64, 65, 2, 66, 67, or 5, preferably the polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 6, 42, 12, 23, 28, 49, 55, 63, 66, 67 or 5. The polypeptide may comprise or consist of the amino acid sequence of any one of SEQ ID NOs: 66, 28-31, 67, 5-9, 42-45, 12-15, 55-58, 23-26, 49-52, 63, 64, 65 or 2, preferably the polypeptide comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 66, 28, 67, 5, 6, 42, 12, 55, 23, 49, or 63.

The polypeptide may have a maximum length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 or 50 amino acids. The C terminal amino acid of the polypeptide may be replaced with the corresponding amide. The polypeptide may comprise a HLA-A2 restricted epitope. The HLA-A2-restricted epitope may comprise or consist of the amino acid sequence of SEQ ID NO: 66 or 67.

The present ~~invention~~disclosure further provides a polynucleotide encoding a polypeptide of the ~~invention~~disclosure. The polynucleotide may be isolated. A vector comprising the polynucleotide is also provided by the present ~~invention~~disclosure.

The present ~~invention~~disclosure also provides a composition comprising a polypeptide of the ~~invention~~disclosure and/or a polynucleotide of the ~~invention~~disclosure and optionally an adjuvant. The composition may further comprise at least one different polypeptide of the ~~invention~~disclosure; at least one different polynucleotide of the ~~invention~~disclosure; and/or at least one pharmaceutically acceptable diluent, carrier or preservative. The adjuvant may be selected from the group consisting of bacterial DNA based adjuvants, oil/surfactant based adjuvants, viral dsRNA based adjuvants, imidazochinilines, and a Montanide ISA adjuvant.

The present ~~invention~~disclosure also provides a method of treating or preventing a disease or condition in a subject, the method comprising administering to the subject a polypeptide of

the ~~invention~~disclosure, a polynucleotide of the ~~invention~~disclosure, and/or a composition of the ~~invention~~disclosure. The method may further comprise the simultaneous or sequential administration of an additional cancer therapy, preferably an antibody.

The present ~~invention~~disclosure also provides a polypeptide of the ~~invention~~disclosure, a polynucleotide of the ~~invention~~disclosure, a composition of the ~~invention~~disclosure, or a combination thereof for use in treating or preventing a disease or condition. The polypeptide, polynucleotide, composition, or combination thereof may be for use in combination with an additional cancer therapy, preferably an antibody.

The present ~~invention~~disclosure further provides use of a polypeptide of the ~~invention~~disclosure, a polynucleotide of the ~~invention~~disclosure, a composition of the ~~invention~~disclosure, or a combination thereof for the manufacture of a medicament for the treatment or prevention of a disease or condition.

The disease or condition may be characterized at least in part by inappropriate or excessive immune suppressive function of TGFb1-expressing cells, and/or wherein the disease or condition is cancer. The disease or condition may be characterized at least in part by inappropriate or excessive expression of interleukin-4 (IL-4) and/or interleukin 13 (IL-13). The disease or condition may be a cancer. Said cancer may be a breast cancer, a cervical cancer, a gastric cancer, a liver cancer, an ovarian cancer, a pancreatic cancer, lung cancer (such as a non-small-cell lung carcinoma (NSCLC)), a melanoma, a leukemia (such as an acute myeloid leukemia (AML)), or a prostate cancer.

The present ~~invention~~disclosure further provides a method of stimulating TGFb1-specific T cells, the method comprising contacting the T cells with a polypeptide of the ~~invention~~disclosure and/or a composition of the ~~invention~~disclosure which comprises at least one polypeptide of the ~~invention~~disclosure. The T cells may be present in a sample taken from a healthy subject or from a cancer patient, optionally a tumour sample.

Brief Description of the Figures

Figure 1A-C. Peptide-specific immune responses in PBMCs from 6 healthy donors were assessed against the array of 38 overlapping 20mer peptides derived from TGFb1 pre-protein by *in vitro* IFNγ ELISPOT assay, set up in triplicate wells. Each spot represents the average number of IFNγ-secreting cells after subtraction of the respective background signal, and the grey horizontal bars indicate the mean values across the tested donors. Stars indicate the peptides that elicited the strongest and the most DFRx2-based statistically significant

CLAIMS

1. A polypeptide which is an immunogenic fragment of human transforming growth factor 1 (TGFb1) and which comprises or consists of a sequence of at least 9 consecutive amino acids of SEQ ID NO: 1.

2. The polypeptide of claim 1, which comprises or consists of up to 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 or 50 consecutive amino acids of SEQ ID NO: 1.

3. The polypeptide of claim 1 or 2, which comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 66, 28-31, 67, 5-9, 42-45, 12-15, 55-58, 23-26, 49-52, 63, 64, 65 or 2.

4. The polypeptide of claim 3, which comprises or consists of the amino acid sequence of any one of SEQ ID NOs: 66, 28, 67, 5, 6, 42, 12, 55, 23, 49, , or 63.

5. The polypeptide of any one of claims 1-4, which has a maximum length of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 or 50 amino acids and/or in which the C terminal amino acid is replaced with the corresponding amide.

6. The polypeptide of any one of claims 1-5, which comprises a HLA-A2 restricted epitope, optionally wherein the HLA-A2-restricted epitope comprises or consists of the amino acid sequence of SEQ ID NO: 66 or 67.

7. A polynucleotide encoding a polypeptide according to any one of claims 1-6, optionally comprised within a vector.

8. A composition comprising a polypeptide according to any one of claims 1-6 and/or a polynucleotide according to claim 7; and optionally an adjuvant.

9. The composition of claim 8 further comprising at least one different polypeptide according to any one of claims 1-6; at least one different polynucleotide according to claim 7; and/or at least one pharmaceutically acceptable diluent, carrier or preservative.

10. The composition of claim 8 or 9, which comprises an adjuvant selected from the group consisting of bacterial DNA based adjuvants, oil/surfactant based adjuvants, viral dsRNA based adjuvants, imidazochinilines, and a Montanide ISA adjuvant.

11. The polypeptide of any one of claims 1-6, the polynucleotide of claim 7, and/or the composition of any one of claims 8-10 for use in therapy.

~~11.~~12. Use of ~~A method of treating or preventing a disease or condition in a subject, the method comprising administering to the subject~~ the polypeptide of any one of claims 1-6, the polynucleotide of claim 7, and/or the composition of any one of claims 8-10 in the manufacture of a medicament for treating or preventing a disease or condition in a subject.

~~12.~~13. The ~~method~~ use of claim ~~11~~12, wherein the disease or condition is:

~~(i)~~ a cancer optionally selected from the group consisting of a breast cancer, a

(i) cervical cancer, a gastric cancer, a liver cancer, an ovarian cancer, a pancreatic cancer, a lung cancer (such as a non-small-cell lung carcinoma (NSCLC)), a melanoma, a leukemia (such as an acute myeloid leukemia), or a prostate cancer; and/or

(ii) characterized at least in part by inappropriate or excessive immune suppressive function of TGFb1-expressing cells and/or inappropriate or excessive expression of IL-4 and/or IL-13.

~~13.~~14. The use~~method~~ of claim ~~11 or 12~~12 or 13, wherein the disease or condition is cancer and the use~~method~~ further comprises that ~~the simultaneous or sequential administration of~~ an additional cancer therapy, preferably an antibody, is to be administered to the subject simultaneously or sequentially.

~~14.~~15. A method of stimulating TGFb1-specific T cells, the method comprising contacting the T cells with: the polypeptide of any one of claims 1-6; and/or a composition of any one of claims 8-10, wherein the composition comprises at least one polypeptide as defined in any one of claims 1-6.

~~15.~~16. The method of claim 15~~14~~, wherein the T cells are present in a sample taken from a healthy subject or from a cancer patient, optionally a tumour sample.