Identification of a HLA-A*0201-restricted immunogenic epitope from the universal tumor antigen DEPDC1

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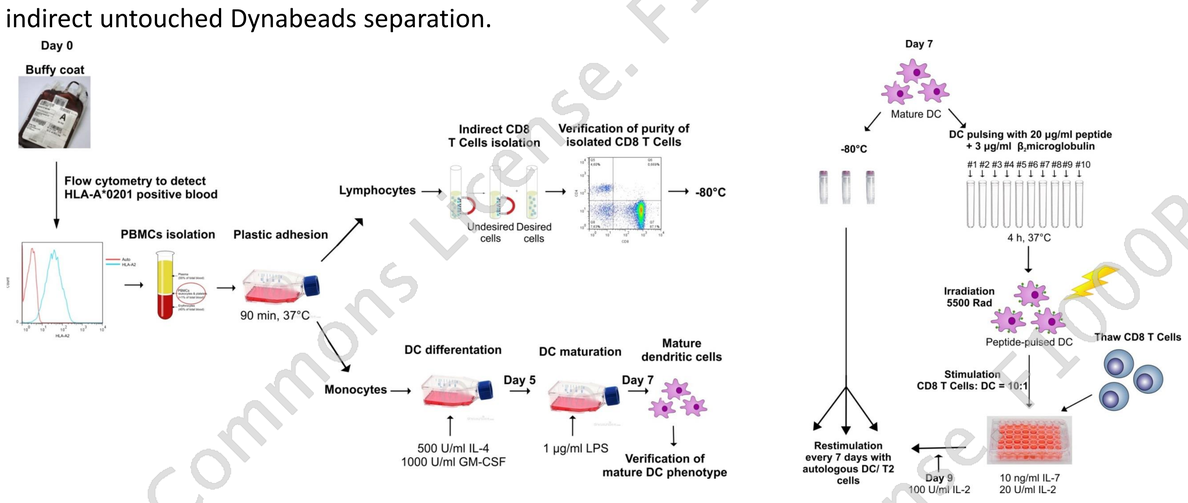
BACKGROUND

With the discovery of tumor-specific and tumor-associated antigens, cancer vaccine strategies as well as adoptive cell therapy with antigen-specific T cells, have become a promising therapeutic approach. Recently, DEP domain containing 1 (DEPDC1) protein has been described to play an important function in cancer cell growth/survival, as its siRNA-mediated knock down suppresses tumor cell growth and increases the number of apoptotic cells (M. Kanehira, et al, 2007), while its overexpression is linked to a bad prognosis in patients with different types of tumors (C. Kretschmer et al, *Mol. Cancer*, 2011; A. Kassambara et al, *PLoS One*, 2013). These data suggest an important involvement of this protein in tumor progression, and therefore its immunological targeting could represent an important strategy to counteract tumor growth and metastasis. A deep search in the Oncomine database confirmed the wide overexpression of DEPDC1 in tumors but its almost complete absence in normal tissues, thus indicating that it can be regarded as an universal tumor antigen and might represent a potential and safe target for immunotherapy. The aim of this study is the identification of an immunogenic DEPDC1-derived peptide HLA-A*0201-restricted (as it is the most common MHC-class I subtype in Caucasian population) to be used for the generation of Ag-specific T cells for adoptive therapy and/or in cancer-vaccination.

MATERIALS AND METHODS

Selection of 9-mer candidate peptides. By integrating the results of BIMAS, NetMHC and NetCTL epitope prediction programs, ten DEPDC1-derived candidate peptides were synthesized, and their capacity to selectively bind and stabilize the HLA-A*0201 molecule was verified using T2 cells, a TAP-deficient HLA-A*0201 positive lymphoblastic cell line.

Generation of *ex vivo* monocyte-derived dendritic cells (DCs) and CD8⁺ T cells. PBMCs from HLA-A*0201 positive healthy donors were isolated by Ficoll gradient centrifugation. The monocyte population, isolated by adhesion on plastic flask, was differentiated into mature DCs by culture for 7 days in presence of IL-4 (500 U/ml) and GM-CSF (1000 U/ml), and by the addiction of LPS (1 µg/ml) for the last 48 hours. The CD8⁺ T cells were isolated from the non-adherent cells and sorted with an indirect untouched Dynabeads separation.



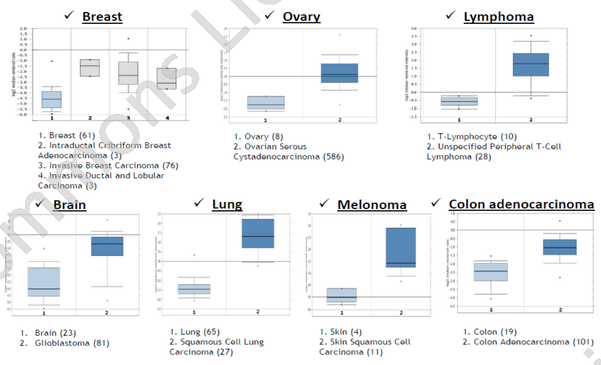
Generation of DEPDC1-derived antigen-specific T cells. Mature DCs were used as APC in the CD8⁺ T cells stimulation protocol. DCs were incubated with 20 µg/ml of different peptides, separately, for 4h at 37°C, irradiated 55 Gy and then incubated with the autologous CD8⁺ T lymphocytes at 1:10 ratio in the presence of IL-7 (10 µg/ml) and IL-2 (20 U/ml). After 48 h, IL-2 was added to the culture at a final concentration of 100 U/ml. Every seven days, the T cells were restimulated with the autologous peptide-pulsed DCs.

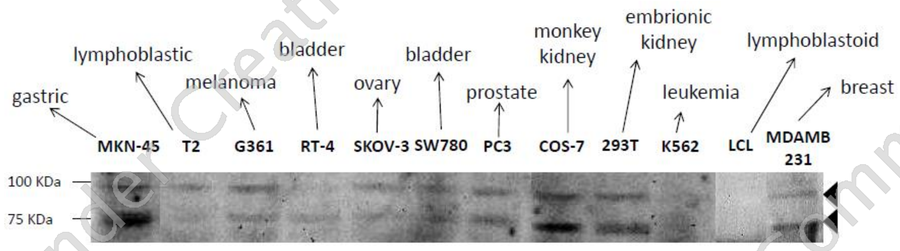
Intracellular cytokine staining (ICS) for IFN-γ detection. An HLA-A*0201 positive lymphoblastoid cell line (LCL) was used as stimulator and was pulsed o/n with 10 µg/ml of peptide, washed and incubated at 1:1 ratio with peptide-specific CTL in presence of an inhibitor of vesicular transports for 6 hours at 37°C. Cells were then stained with anti-CD8 mAb, washed and fixed. After permeabilization, cells were stained with anti-IFN-γ mAb and flow cytometry analysis was performed.

Cytotoxicity assay. After 4 stimulations, the reactivity of peptide-specific CTL generated was tested against HLA-A*0201 positive cancer cell lines expressing DEPDC1 endogenously, using a 6-hours ^{51}Cr-release assay. The percentage of specific lysis was calculated as follows: % specific lysis = (specific ^{51}Cr release – spontaneous ^{51}Cr release)/(maximal ^{51}Cr release – spontaneous ^{51}Cr release) x 100.

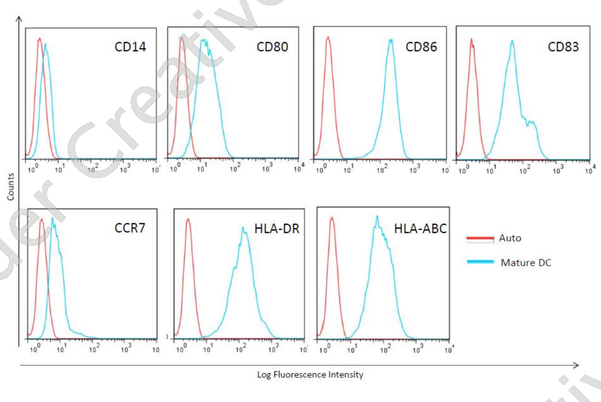
RESULTS

DEPDC1 overexpression in tumor cell lines. We confirmed by a deep analysis in Oncomine and further by western blot analysis, that DEPDC1 is overexpressed in different cancer cell lines, such as gastric, melanoma, ovary, breast, prostate and bladder cells, but is not expressed in normal tissues.

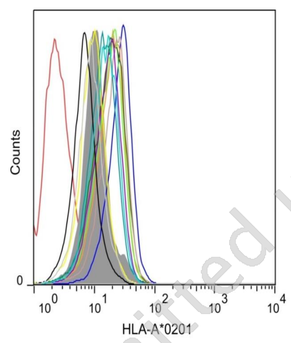




Generation and maturation of DCs. The surface expression of CD80, CD86, CD83, HLA-ABC, HLA-DR, CCR7, and the down regulation of CD14 in the phenotypic flow cytometry analysis confirmed the maturation of DC.

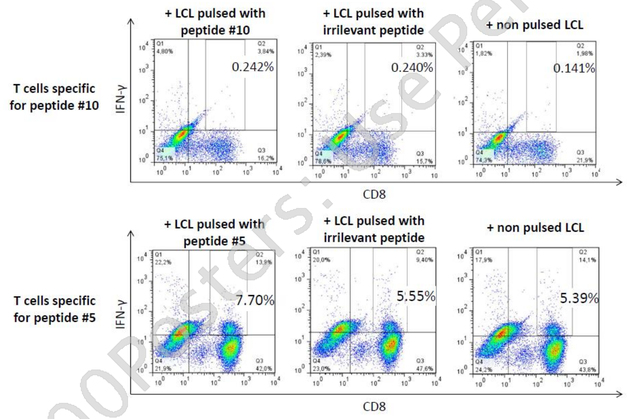


HLA-A*0201 stabilization on T2 cells surface. We verified by a stabilization assay using TAP-deficient T2 cells, the binding of the DEPDC1-derived candidate peptides to HLA-A*0201, especially for the number 1 and 5 peptides, in contrast to a control peptide with no predicted binding.

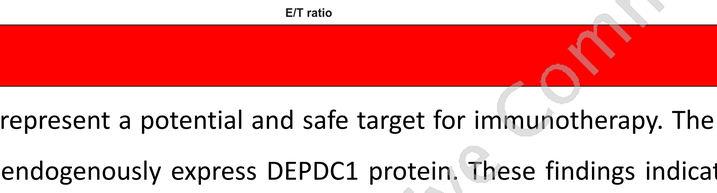


Sample Name	Mean , FL1-H
T2 auto	3,41
T2 alone (gray fill)	12,5
Peptide # 1	29,2
Peptide # 2	20,6
Peptide # 3	19,7
Peptide # 4	19,8
Peptide # 5	23,1
Peptide # 6	18,0
Peptide # 7	15,7
Peptide # 8	10,5
Peptide # 9	16,6
Peptide # 10	9,73
Peptide ctrl -	7,71

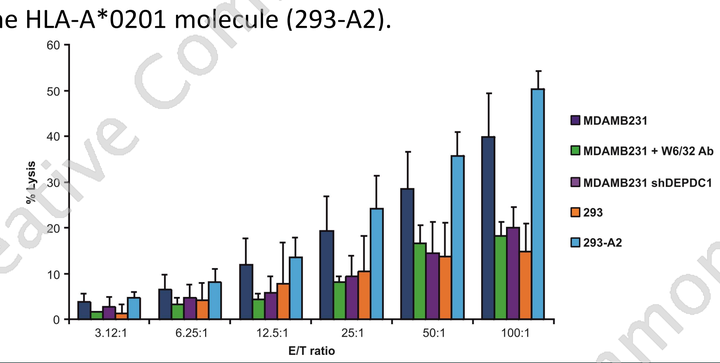
HLA-A*0201 #5 is the candidate peptide. Peptide #5-specific CTL produced IFN-γ when cocultured with LCL pulsed with the DEPDC1-derived peptide #5 among all of the candidate peptides. The high level of IFN-γ production in response to LCL left untreated or pulsed with an irrilevant peptide is due to a high sequence homology between the peptide #5 and different EBV-derived epitopes, as revealed by BLAST alignment.



CTL phenotype characterization. Overall, more than 50% of cells stimulated with autologous DC pulsed with DEPDC1-derived peptide #5 were CD3⁺CD8⁺ T cells (54,1% ± 2,55%) and about 8% were CD4⁺ T cells (7,64% ± 5,87%). Most of the CD8⁺ T cells (77,6% ± 5,31%) had an effector memory profile (CD45RA⁻CCR7⁻).



Peptide #5-specific CTL displays cytotoxic activity against HLA-A*0201 DEPDC1 positive cell lines. The peptide #5-specific CTL cells were able to kill the triple negative breast cancer cell line MDAMB231, which is HLA-A*0201 positive and endogenously expresses DEPDC1. No cytotoxicity was observed against MDAMB231 cells where DEPDC1 was silenced by siRNA (MDA shDEPDC1). The presence of an anti-MHC class I blocking antibody (W6/32) in the assay demonstrated the MHC class I-restricted nature of the lysis mediated by the CTL, as well as no lysis was observed against HLA-A*0201⁻DEPDC1⁺ 293 cell line. As expected, the cytotoxic activity was restored by using 293 cells modified to express the HLA-A*0201 molecule (293-A2).



CONCLUSIONS

The selective DEPDC1 overexpression in cancer cells indicates that it can be regarded as an universal tumor antigen and might represent a potential and safe target for immunotherapy. The CTL populations generated using the selected peptide present a fine specificity, HLA-A*0201 restriction and the ability to kill tumor cells that endogenously express DEPDC1 protein. These findings indicate that this HLA-A*0201-restricted DEPDC1-derived peptide is a putative tumor antigen that could be exploited for vaccination against different tumors overexpressing the DEPDC1 protein.