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CAR's made it to the pancreas

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Despite intensive treatment, pancreatic adenocarcinoma still has the worst prognosis among all malignancies. Using clinically relevant models, we demonstrated the therapeutic efficacy of adoptively transferred T cells engineered with a carcinoembryonic antigen (CEA)- and ERBB2-specific chimeric antigen receptor against pancreatic carcinoma. Targeting CD24, a putative cancer stem cell antigen expressed by a minority of carcinoma cells, was likewise effective.

Keywords: adoptive cell therapy, cancer stem cell, chimeric antigen receptor, pancreas adenocarcinoma, T cell

Abbreviations: ACT, adoptive cell therapy; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen

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Pancreatic adenocarcinoma is often diagnosed at an advanced stage with a mean survival rate of less than 2 years and no primary therapy. In this situation, adoptive cell therapy with patient's cytotoxic T cells redirected toward tumor-associated antigens may provide a therapeutic option. The "T-body" strategy pioneered and developed by our groups[1] is based on engineering T cells with a chimeric antigen receptor (CAR) with antibody-defined specificity for a target expressed on cancer cells.[2] Such genetically modified T cells showed remarkable efficacy against leukemia in recent trials,[3] but their efficacy against solid neoplasms had not yet been investigated.[4]

We have addressed this issue in a clinically relevant model of carcinoembryonic antigen (CEA) expression, which closely mimics the human situation with respect to physiological expression of the targeted antigen on healthy tissues, presence of the antigen in serum and competence of the immune system.[5,6] Among several proteins, pancreatic carcinoma cells overexpress CEA and ERBB-2 (HER2/neu), which are not tumor-specific but also expressed on epithelial cells of healthy tissues like lungs and the colon, although to a lesser degree. Adoptive therapy with CEA-specific T cells harbors the risk of damaging healthy CEA$^+$ tissues, resulting in auto-immune reactions, as observed in a recent trial that is on hold due to severe auto-immune colitis and pneumonia.[7]

The CEA-transgenic mouse expresses CEA in the gastrointestinal and pulmonary tract, secretes soluble CEA into the serum and is self-tolerant toward CEA (**Fig. 1**). Mouse pancreatic adenocarcinomas with or without CEA expression were treated by the adoptive transfer of murine T cells engineered with an anti-CEA CAR. All mice showed profound regression of CEA$^+$ pancreatic cancer, and six out of eight mice achieved sustained remissions. The antitumor response was CEA-specific and CAR-mediated.

We made several observations that add to our understanding of adoptive T-cell therapy:

(1) Following systemic administration, anti-CEA CAR T cells accumulated at the site of CEA$^+$ pancreatic tumors, without inducing severe colitis nor infiltrating to major extents into the colon or lungs, although these tissues physiologically express CEA. Lack of autoimmune activation may be due to the fact that the epithelia of healthy tissues, as opposed to carcinoma cells, express CEA in a polarized fashion (to the lumen), hence making it less accessible to infiltrating T cells.

(2) The CAR that we used has a moderate binding affinity, i.e., 37 nM, that is lower than that of CARs used in clinical trials. CARs of different affinity differ in their activation threshold. Those of high affinity might lose the selectivity

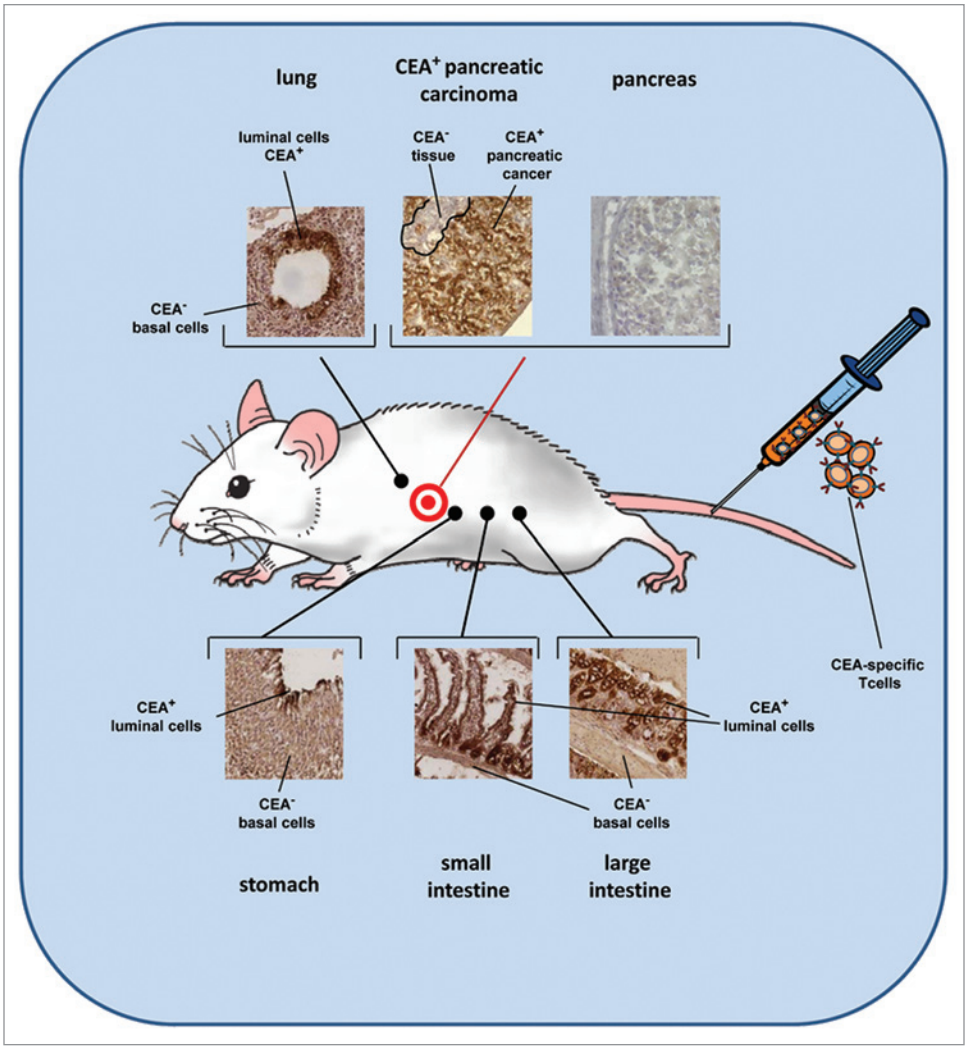

Figure 1. Adoptive cell therapy of pancreas carcinoma in a pre-clinical mouse model. The carcinoembryonic antigen (CEA)-transgenic mouse expresses CEA on luminal epithelial cells of the gastrointestinal tract and the lung, mimicking the human expression pattern. CEA+ pancreatic adenocarcinoma is established in the pancreas by transplantation of tumor cells. Once the carcinoma is established, autologous T cells engineered with a CEA-specific chimeric antigen receptor are adoptively transferred by i.v. injection. Engineered T cells eliminate CEA+ pancreatic carcinoma without inducing autoimmune pathology.

for cells with a high antigen load and may hence cause a greater degree of auto-immunity.

(3) The role of lymphodepletion, which is normally performed to facilitate the homeostatic expansion of transferred T cells, in causing autoimmune pathology remains unclear. In our mouse model, lymphodepletion was neither required for achieving therapeutic efficacy nor did it result in auto-immunity.

(4) T-cell targeting to the tumor site occurred even in presence of soluble CEA at clinically relevant concentrations. Serum CEA binds to but does not activate engineered T cells, unless CEA is present in micro-domains on the cell surface to induce CAR clustering and formation of a signaling synapse.[8]

(5) Adoptively transferred CAR-expressing T cells had a central memory phenotype and became activated at the targeted tumor site. Nevertheless, they converted to an exhausted phenotype after prolonged persistence. Strategies to prevent exhaustion still need to be explored.

(6) Mice that successfully eliminated CEA+ carcinoma also rejected a secondary tumor cell challenge in a CEA-specific fashion, in line with previous observations obtained in other models.[9] The process is accompanied by a humoral and a T_H1 dominated CD4+ T-cell response,[5] implying that T-bodies may act as mediators for a helper T cell-dependent humoral response.

(7) The observations made in the pancreatic adenocarcinoma model cannot be extended to colon carcinoma, a setting in which adjacent healthy CEA+ epithelial cells may become accessible to T cells, thereby increasing the risk of auto-immunity.

In a second report,[6] we explored the selective elimination of "cancer stem cells," rather than of bulk tumor cells, by targeting CD24 expressed on putative adenocarcinoma stem cells. Anti-CD24 CAR-expressing T cells could successfully eliminate orthotopic human pancreatic adenocarcinoma xenografts in which

$CD24^+$ tumor cells represent a minority. These xenografts resist final elimination by anti-ERBB2 T-bodies, in contrast to xenografts in which all cells expressed ERBB2. The treatment was highly effective in reducing the size of primary tumors, in particular in situations of high tumor burden, and in eliminating remote metastases.

The significant lesson of this study consists in the impact of CAR specificity. Targeting ERBB2, which is broadly, but not uniformly, expressed by pancreatic adenocarcinoma cells, did not enduringly eradicate tumors whereas targeting CD24, which is expressed by a minority of cancer cells, arrested growth of freshly established xenografts. $CD24^-$ tumor cells may represent more mature cancer cells without stem cell capacities, and hence unable to maintain tumor progression once $CD24^+$ cells are eliminated from the lesion. This observation supports the cancer stem cell hypothesis and is consistent with a recent report demonstrating antitumor activity of CAR-expressing T cells redirected against a cancer stem cell antigen other than CD24.[10]

Additionally, this study shows that an adoptive treatment with a relatively low dose of CAR-engineered T cells cleared most of the tumor burden within a week. Additional boosts can be administered as needed, using cells bearing CARs with the same or different specificities. Such protocols with a relatively low T-cell dose reduce the risk of a massive release of pro-inflammatory cytokines, the life-threatening condition known as "cytokine storm." Moreover, the alternate use of different CAR specificities for each course of treatment may overcome the emergence of escape variants.

Another lesson learnt from this study is that the intra-tumor administration of T-bodies, which is a clinical option for patients with localized, endoscopically accessible pancreatic cancer, completely eradicated the neoplastic lesion. The procedure may be safer than systemic T cell administration, especially when the antigen targeted by T cells is also expressed on essential organs.

Taken together, our studies are the first to demonstrate, in clinically relevant models, that CEA-redirected T cells can specifically and efficiently eliminate CEA+ pancreatic carcinomas without inducing substantial auto-immunity. Targeting putative cancer stem cells proved equally effective, although such cells represent a minority of the tumor mass. Both our studies provide a rationale for the adoptive T-cell therapy of this currently untreatable tumor.

References

1. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci U S A 1993; 90:720-4; PMID:8421711; http://dx.doi.org/10.1073/pnas.90.2.720.
2. Eshhar Z. The T-body approach: redirecting T cells with antibody specificity. Handb Exp Pharmacol 2008; 181:329-42; PMID:18071952; http://dx.doi.org/10.1007/978-3-540-73259-4_14.
3. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 2011; 365:725-33; PMID:21830940; http://dx.doi.org/10.1056/NEJMoa1103849.
4. Gilham DE, Debets R, Pule M, Hawkins RE, Abken H. CAR-T cells and solid tumors: tuning T cells to challenge an inveterate foe. Trends Mol Med 2012; 18:377-84; PMID:22613370; http://dx.doi.org/10.1016/j.molmed.2012.04.009.
5. Chmielewski M, Hahn O, Rappl G, Nowak M, Schmidt-Wolf IH, Hombach AA, et al. T cells that target carcinoembryonic antigen eradicate orthotopic pancreatic carcinomas without inducing autoimmune colitis in mice. Gastroenterology 2012; 143:1095-107, e2; PMID:22750462; http://dx.doi.org/10.1053/j.gastro.2012.06.037.
6. Maliar A, Servais C, Waks T, Chmielewski M, Lavy R, Altevogt P, et al. Redirected T Cells that target pancreatic adenocarcinoma antigens eliminate tumors and metastases in mice. Gastroenterology 2012; 143:1375–84, e5; PMID:22819865; http://dx.doi.org/10.1053/j.gastro.2012.07.017.
7. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther 2011; 19:620-6; PMID:21157437; http://dx.doi.org/10.1038/mt.2010.272.
8. Hombach A, Koch D, Sircar R, Heuser C, Diehl V, Kruis W, et al. A chimeric receptor that selectively targets membrane-bound carcinoembryonic antigen (mCEA) in the presence of soluble CEA. Gene Ther 1999; 6:300-4; PMID:10435115; http://dx.doi.org/10.1038/sj.gt.3300813.
9. Chmielewski M, Rappl G, Hombach AA, Abken H. T cells redirected by a CD3ζ chimeric antigen receptor can establish self-antigen-specific tumour protection in the long term. Gene Ther 2012; In Press; PMID:22378346; http://dx.doi.org/10.1038/gt.2012.21.
10. Schmidt P, Kopecky C, Hombach A, Zigrino P, Mauch C, Abken H. Eradication of melanomas by targeted elimination of a minor subset of tumor cells. Proc Natl Acad Sci U S A 2011; 108:2474-9; PMID:21282657; http://dx.doi.org/10.1073/pnas.1009069108.