Immuno-targeting of pancreatic cancer stem cells
A new therapeutic strategy against a devastating disease?

Michele Cioffi and Christopher Heeschen*

Keywords: pancreatic cancer, cancer stem cells, bispecific antibody, xenograft, CD133, EpCAM

Abstract: Pancreatic ductal adenocarcinoma (PDAC), the most frequent form of pancreatic cancer, is the deadliest solid cancer and currently the fourth most frequent cause of cancer-related deaths. PDAC is characterized by late diagnosis due to lack of early symptoms, extensive metastasis, and high resistance to chemotherapy and radiation. Despite expanding research activities in the field of pancreatic tumor and vascular biology, there has been little therapeutic progress regarding clinical endpoints over the past decades. Since the 1990s, the anti-metabolite gemcitabine emerged as the gold standard for treatment of patients with PDAC but with a 5-y survival rate of 1–4% and a median survival period of 4–6 mo, the prognosis of patients with advanced PDAC remains extremely poor.

Since the establishment of the cancer stem cell (CSC) hypothesis for leukemia in 1994, convincing evidence has also emerged for solid tumors that, like adult tissues, are sustained and promoted by cells that exhibit features of stem cells such as unlimited self-renewal capacity. We and others have recently provided conclusive evidence for a hierarchal organization of human PDAC and, even more importantly, demonstrated that pancreatic CSCs at the top of the hierarchy are driving metastasis and are resistant to chemotherapies.

Initial studies from our groups are now providing increasing evidence that direct targeting of pancreatic CSCs in combination with elimination of the more differentiated tumor cells bears therapeutic value as this significantly prolonged survival in preclinical xenograft models. Several antigens have been identified in the past years to target tumor cells. A recent study by Visus and colleagues took advantage of the ALDH activity as a marker to identify and selectively target the CSC population using several cell lines including pancreatic cancer cells. The authors generated in vitro ALDH1A1-specific CD8+ T cells in order to eliminate ALDH1A1+ CSC in preclinical models of human tumor xenografts and observed growth inhibition and reduced metastasis. However, a major concern for this approach represents the fact that ALDH1A1-specific CD8+ T cells will most likely also target normal ALDH1A1+ stem cells, which can for example be found in the hematopoietic system.

In our recent work, we evaluated the therapeutic value of the bispecific antibody MT110 targeting the T-cell receptor CD3 complex and Epithelial cell adhesion molecule (EpCAM; CD326). EpCAM is frequently overexpressed and functionally

*Correspondence to: Christopher Heeschen; Email: christopher.heeschen@cnio.es

Submitted: 01/13/12; Accepted: 01/13/12

http://dx.doi.org/10.4161/onci.19368

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; CSC, cancer stem cell; EpCAM, epithelial cell adhesion molecule; PBMC, peripheral blood-derived mononuclear cells

Pancreatic cancer is a highly aggressive and deadly disease harboring a distinct population of cancer stem cells (CSCs) that is not affected by conventional therapies. A new therapeutic approach using the EpCAM/CD3-bispecific antibody MT110 is capable of activating and redirecting cytotoxic T cells to eliminate primary human pancreatic cancer stem cells, which resulted in long-term survival of preclinical xenografts models.
altered in epithelial cancer cells, including CSC, and therefore is becoming accessible on the surface of these cells. In contrast, in normal epithelial cells and embryonic stem cells, EpCAM is sequestered within intercellular boundaries. Therefore, EpCAM represents a promising target for immunotherapy of EpCAM-expressing cancer cells including tumorigenic CSC.

We first evaluated the effect of MT110 using a dose escalation and time dependent approach in three different primary PDAC cells isolated from human cancer tissue samples. We observed that T cells reach maximal activity for inducing apoptosis of cancer cells at a concentration of 100 ng/ml MT110, as demonstrated by flow cytometry analysis for early T-cell activation marker CD69 and late activation marker CD25. Subsequent flow cytometry analysis for CSC markers identified by expression of CD133 and SSEA1, respectively, showed a significant reduction in the CSC population implying that they are not spared from the cytotoxic activity of activated T cells. Moreover, as a surrogate assay for the self-renewal capacity of CSCs, we examined the sphere formation capacity of the cells following treatment with MT110. As predicted, primary cancer cells exposed to MT110 for 7 d showed a significant decline in sphere formation.

The most defining feature of CSC is their ability to exclusively form tumors in vivo. Indeed, CSC treated for 7 d with MT110 and then implanted into mice had completely lost their in vivo tumorigenicity. Finally and most importantly, we then studied the treatment effects of MT110 in vivo using a model of established primary human PDAC co-implanted with healthy donor-derived PBMC. After administration of MT110 we observed a complete stall in tumor growth over the entire follow-up period indicating that the tumors were depleted for tumorigenic/tumor-promoting CSC. This was also confirmed by flow cytometry analysis of harvested tumors, which revealed a depletion for cells expressing CD133, SSEA-1, and CXCR4 as well as significantly reduced sphere-formation capacity in the MT110 group as compared with tumors harvested from mice treated with control BiTE.

MT110 is currently tested in a dose-escalating Phase I clinical trial enrolling patients with diverse epithelial cancers (lung, colon, gastric). Low toxicity and early signs of biological activity have been observed at clinically well-tolerated doses of MT110 in this first-in-human clinical trial. Our results derived from preclinical PDAC models now suggest that this treatment regimen could also represent a new opportunity for patients with PDAC. It is important to note, however, that the strong fibroblastic nature of PDAC results in poor tumor vascularization and may therefore require simultaneous targeting of the stroma, e.g., by the addition of hedgehog pathway inhibitors for sufficient delivery of MT110 to the cancer cells including the CSC subpopulation.
References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60:27-40; PMID: 20610543; http://dx.doi.org/10.3322/caac.20077.

2. Marano E, Tagliabue F, Libri A, Diamanti V, Fabbrocini A, De Lorenzo S, et al. Gemcitabine combined with continuous infusion 5-fluorouracil in advanced and symptomatic pancreatic cancer: a clinical benefit-oriented phase II study. Br J Cancer 2000; 82:1772-5; PMID:11009389; http://dx.doi.org/10.1054/bjoc.1999.1139.

3. Lapidot T, Strad C, Virmesse J, Manchot R, Huang T, Goujon C, et al. A cell initiating human acute myeloid leukemia after transplantation into SCID mice. Nature 1994; 367:645-8; PMID:7509044; http://dx.doi.org/10.1038/367645a0.

4. Hermann PC, Huber S, Horike T, Aicher A, Elsholtz PE, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 2007; 1:153-63; PMID:17873365; http://dx.doi.org/10.1016/j.stem.2007.04.062.

5. Muller MT, Hermann PC, Wahauer J, Robin-Voyette B, Leslie SR, Halter S, et al. Combined surgical treatment to eliminate tumorigenic cancer stem cells in human pancreatic cancer. Gastroenterology 2009; 137:1110-3; PMID:19501596; http://dx.doi.org/10.1054/gastro.2009.05.067.

6. Lapidot T, Hermann PC, Muller MT, Halter S, Bille A, Miranda-Leon A, et al. Nodal/Activin signaling drives self-renewal and tumorigenicity of pancreatic cancer stem cells and provides a target for combined drug therapy. Cell Stem Cell 2011; 9:633-46; PMID:21950644; http://dx.doi.org/10.1016/j.stem.2011.10.001.

7. Visus C, Wang Y, Luason-Leon A, Friesen HL, Silver S, Scarpellini ML, et al. Targeting ALDHbright human carcinoma-initiating cells with ALDH1A1-specific CAR T cells. Clin Cancer Res 2011; 17:6778-89; PMID:21896799; http://dx.doi.org/10.1158/1078-0432.CCR-11-1131.

8. Cioffi M, Dorade J, Bencard PA, Hahnfeld C. EpCAM+ CDO-Bispecific T-cell Engaging Antibody. M1110 Eliminates Primary Human Pancreatic Cancer Stem Cells. Clin Cancer Res 2012; 18:4641s; PMID:22996026; http://dx.doi.org/10.1158/1078-0432.CCR-11-1278.

9. Mirm M, Bencard PA, Gross O. The emerging role of EpCAM in cancer stem cell signaling. Cancer Res 2009; 69:5627-8; PMID:19584271; http://dx.doi.org/10.1158/0008-5472.CAN-09-0654.

10. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133:704-15; PMID:18485877; http://dx.doi.org/10.1016/j.cell.2008.03.027.