Chemotherapy:Open Access

Principe, et al., Chemo Open Access 2016, 5:2
http://dx.doi.org/10.4172/2167-7700.1000188

Commentary Open Access

Blockade of Surface Alpha-Enolase (ENO1) as a Novel Immunotherapeutic Approach in Pancreatic Cancer

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Received date: January 11, 2016; **Accepted date:** February 15, 2016; **Published date:** February 20, 2016

Keywords: Pancreatic cancer; ENO1; Antibody; Immunotherapy

Introduction

In 2010 in Italy, there were 175,046 cancer deaths, with total mortality rates, respectively for men and women, of 138 and 83/100,000 [1]. Total cancer mortality in Italy has been declining since the 1980s in men and earlier in women [1], similarly to most European countries [2] and the United States [3]. However, some rising trends have also been observed, notably for female lung cancer and pancreatic cancer in both sexes [1]. Although the incidence of pancreatic cancer is relative low compared to other tumor types such as breast, lung and prostate cancer, in pancreatic cancer the mortality is almost equal to the incidence level, with 41,780 and 40,560 deaths predicted in Europe and USA respectively [2,4]. For this reason, PDA is the 4th leading cause of cancer deaths in the Western Countries [1,2,4]. Despite the efforts of clinicians and scientists, pancreatic cancer has the worst prognosis of all major malignancies, with a 5-year survival rate of 8% [4].

Pancreatic ductal adenocarcinomas (PDA), originate from the exocrine pancreas, account for 95% of pancreatic cancers and is characterized by rapid progression, invasiveness and resistance to both radiation and chemotherapy [5]. Therefore, surgery provides the only possibility for cure, but most patients present metastatic PDA upon initial diagnosis, and less than 20% of patients are suitable candidates at the time of diagnosis and 5-years survival of completely resected patients is only up to 25% [5]. The major hurdle for PDA is the lack of markers for early diagnosis, along with few efficacious therapies.

Alpha-Enolase (Eno1) and Anti-Eno1 Antibody-Based Immunotherapy

In recent years, targeted therapies have been tested for treating metastatic pancreatic cancer, for example Erlotinib, an inhibitor of the epidermal growth factor receptor (EGFR) (ClinicalTrials.gov number NCT01608841), is a successful example of how using an antibody, in combination with gemcitabine-based chemotherapy, is effective in improving overall and progression-free survival, as well as response rates in metastatic PDA patients [6].

Immunotherapy, by both passive and active approaches, can be considered as a kind of "targeted therapy" in terms of activating the immune system against a specific target, or nullifying it through an antibody. We have previously identified novel PDA-associated antigens by detecting autoantibodies in the sera of PDA patients, which can be considered as "spies" of the activation of the immune system [7]. Some of these antigens have been characterized as suitable therapeutic targets, such as α-Enolase (ENO1) [8]. ENO1 is a multi-functional protein, acting as both a glycolytic enzyme and a plasminogen receptor expressed on the cell surface of tumor cells, thus playing a critical role in metastasis and spreading [9]. As glycolytic enzyme is ubiquitously expressed in almost normal tissues (see website www.proteinatlas.org) including liver, brain, spleen, adipose tissue, kidney [10], reproductive tract [11] and pancreas [8]. By promoting plasminogen activation into plasmin, a serine-protease involved in extracellular matrix degradation, ENO1 favors cell invasion and metastasis (Figure1 upper panel) [9]. The plasminogen system is involved in tumor growth, invasion and metastasis [12]. Several proteins of this system have been demonstrated to have a clinical value as diagnostic and prognostic markers in cancer. In particular, overexpression of plasminogen receptors has been associated with poor prognosis, shorter survival and resistance to chemotherapy [12].

We first characterized ENO1 as a plasminogen receptor; it is expressed on the surface of human PDA cells lines and at higher levels in metastatic cell lines, whereas it is absent or expressed at lower levels in primary tumor-derived cell lines [13]. *Ex vivo* analysis of a liver metastasis-derived cell line (PANC-1/M) revealed an increased cell surface expression of ENO1 compared to the parental cell line (PANC-1/P) from the primary tumor. This observation suggests that spreading and invasion of PDA cells is strictly related to the high cell surface expression of ENO1, which, in turn, facilitates binding of elevated concentrations of plasminogen at the cell surface. Plasminogen is cleaved into plasmin by uPA (urokinase plasminogen activator), resulting in an increased ability of PDA cells to degrade the ECM [12]. We targeted surface ENO1 by using a mouse monoclonal antibody (mAb) that blocks the plasminogen-ENO1 interaction [13]. The anti-human ENO1 mAb inhibited plasminogen-dependent cell migration through Matrigel *in vitro*. This invasion requires simultaneous expression of surface ENO1 and uPAR (receptor for uPA), as well as expression of uPA. Although uPA and uPAR were not expressed in PDA cells, *in vivo* they can be expressed by tumor stromal cells that are abundantly present in the PDA microenvironment, or up-regulated in cancer cells by growth factors (such as EGF and HGF) or cytokines (such as TNF-α, TGF-β) [14-17]. In fact, exposure of ENO1-expressing PDA cells to TGF-β induced the up-regulation of uPA and uPAR, which renders the cells sensitive to plasminogen in terms of increased invasion. *In vivo*, immune-compromised mice treated with anti-ENO1 mAb displayed a reduced number of metastasis after xeno-transplantation of PDA cells compared to control IgG-treated mice

[13]. These data indicate that ENO1 is involved in the PDA invasion process, suggesting that interfering with the ENO1-plasminogen interaction could be useful in inhibiting this invasion. The anti-ENO1 mAb effect is strictly related to this interaction, as demonstrated by *in vitro* and *in vivo* experiments, using PDA cells expressing a triple-mutated plasminogen binding site of ENO1 (CFPAC-1 TM). These cells were no longer able to invade in response to plasminogen.

An innovative Adeno Associated Viral Vector (AAVV)-based therapeutic strategy has been adopted to increase the efficacy of anti-ENO1 mAb treatment in a metastatic mouse model. This approach exploited the innovative technology of antibody gene transfer [18-19]. Passive immunization involves the delivery of purified antibodies to the host with a resulting short-lived immunity, necessitating numerous inoculations to produce an appreciable effect. By contrast, when the mAb gene is packaged into an (AAVV) vector and delivered by direct intramuscular injection, the mAb molecules are endogenously synthesized and passively distributed to the circulatory system, thus providing long-term protection. This strategy has been proved to be successful. After injection of the AAVV expressing anti-ENO1 mAb (Figure 1 left panel), the presence of the mAb in the mouse sera progressively increased from 1 to 4 weeks after injection. This continuous, long-lasting and sustained production of circulating anti-ENO1 mAb resulted in the observation of a significant decrease in lung metastases compared to control mice, producing a more pronounced effect compared to biweekly inoculation of anti-ENO1 mAb (Figure 1 left panel)[13].

Taken together, these findings strongly suggest that blocking the ENO1/plasminogen interaction through the injection of AAVV-anti-ENO1 mAb could provide a new therapeutic approach for treating metastatic PDA patients.

Natural Anti-ENO1 Antibodies Elicited By ENO1 Vaccination

We have also demonstrated that ENO1 is able to induce both humoral and T cell specific responses in PDA patients [20]. Autoantibodies against ENO1 are detected in more than 60% of PDA patients, and their presence correlates with a longer survival [7-8]. This prompted us to propose ENO1 as a therapeutic candidate owing to its ability to induce an integrated humoral and cellular response. We developed a DNA vaccination to ENO1 in genetically engineered mice (GEM) that spontaneously develop autochthonous, lethal pancreatic cancer [21], and we showed that the vaccine significantly induced a specific immune response against pancreatic tumors, which efficiently prolonged mouse survival. We demonstrated that ENO1 DNA vaccination induced a specific antibody as well as a cellular response in mice. Notably, DNA vaccination elicited anti-ENO1 IgG binding at the surface of GEM-derived PDA cells, which killed them by inducing a complement-dependent cytotoxicity (Figure 1 central panel) [21]. Furthermore, ENO1 DNA vaccination reduced the numbers of peripheral and intratumoral myeloid-derived suppressor cells (MDSC) and T-regulatory cells and increased T-helper 1 (Th1) and Th17 responses. Due to the ENO1 cell surface expression on MDSC, and its enhancement by inflammation, we also investigated if anti-ENO1 antibodies interfered with MDSC invasion in pancreatic tumors [22]. *In vitro*-generated MDSC treated with anti-ENO1 antibodies were less able to adhere to pancreatic endothelial cells and invade them or the Matrigel (Figure1 right panel). In addition, there was significantly less migration of MDSC treated with anti-ENO1 to the lymph node after foot-pad injection. Finally, ENO1-vaccinated mice showing anti-ENO1 specific antibodies, displayed a lower percentage of MDSC in the tumor compared to that evaluated in empty-plasmid vaccinated mice [22].

Despite ENO1 is found in almost all human tissues [23], the increased expression of ENO1 and its cell surface localization is a unique feature of cancer cells. ENO1-specific T cells spared normal keratinocytes in *in vitro* cytotoxic assay [20] and no autoimmunity event nor side effects were detected after DNA-vaccination [21] or anti-ENO1 mAb treatment [13] in mice. This suggests that both ENO1-DNA vaccine and anti-ENO1 mAb therapy are safe.

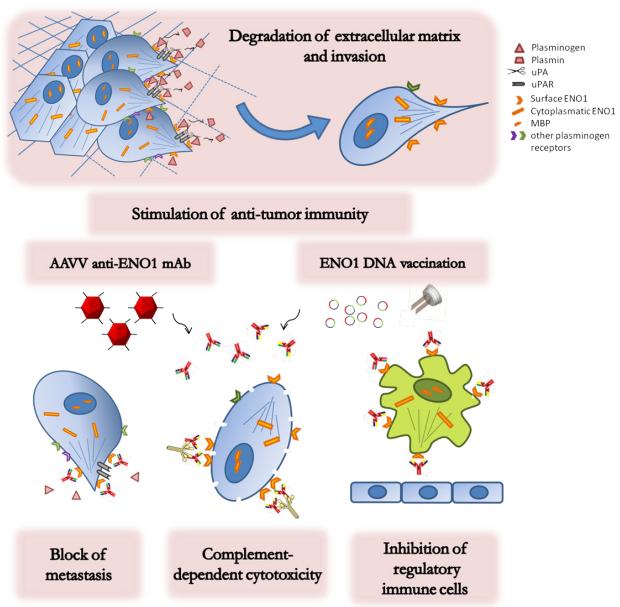

Figure 1: Anti-ENO1 antibody immunotherapy. The plasminogen receptor ENO1 causes plasminogen to localize to the extracellular surface, allowing its cleavage into plasmin, catalyzed by uPA bound to its surface receptor uPAR. Plasmin is a serine protease that degrades extracellular matrix (ECM) proteins and helps the invasive process. The anti-ENO1 antibodies, produced using the AAV construct or elicited by the vaccination, inhibit the binding of plasminogen to ENO1 or binding to the endothelial cells, blocking invasion of both tumor cells and MDSC. Anti-ENO1 treated MDSC also favor Th1 and Th17 cell responses. In addition, anti-ENO1 Abs mediate complement-dependent cytotoxicity and PDA cell killing.

Conclusion

Metastasis is one of the greatest clinical challenges in modern oncology; it is, in fact, the disseminated disease that causes the death of cancer patients. This is especially true for PDA, which is one of the most aggressive human cancers, due to its high potential of local invasion and metastasis [5]. Despite many studies, metastasis remains largely a terra incognita waiting to be explored. Our recent findings highlight how the anti-ENO1 antibodies inhibit PDA cells, as well as suppressor immune cells, making the immunotherapy effective (Figure 1). Taken together, these results once again define ENO1 as a promising molecular and immunological target for the realization of new personalized therapies for PDA patients.

Acknowledgements

We would like to thank Dr. Radhika Srinivasan for critically reading the manuscript. This work was partly supported by grants from the Associazione Italiana Ricerca sul Cancro (Special Program 5 x mille no. 12182 to FN and IG no. 15257 to FN); University of Turin-Progetti Ateneo 2014-Compagnia di San Paolo (PC-METAIMMUNOTHER to FN; PANTHER to PC); Ministero della Salute, Progetti di Ricerca Sanitaria Finalizzata 2015 (RF-2013-02354892); Fondazione Ricerca Molinette; MP is recipient of a fellowship from Fondazione Ursula Giorgio Cytron.

References

1. Rosso T, Bertuccio P, La Vecchia C, Negri E, Malvezzi M, et al. (2015) Cancer mortality trend analysis in Italy, 1980-2010, and predictions for 2015. Tumori 101: 664-675.
2. Malvezzi M, Bertuccio P, Rosso T, Rota M, Levi F, et al. (2015) European cancer mortality predictions for the year 2015: does lung cancer have the highest death rate in EU women? Ann Oncol 26: 779-786.
3. Chatenoud L, Bertuccio P, Bosetti C, Malvezzi M, Levi F, et al. (2014) Trends in mortality from major cancers in the Americas: 1980-2010. Ann Oncol 25: 1843-1853.
4. Siegel RL, Miller KD, Jemal A (2016) Cancer statistics, 2016. CA Cancer J Clin 66: 7-30.
5. Kamisawa T, Wood LD, Itoi T, Takaori K (2016) Pancreatic cancer. Lancet .
6. Wang JP, Wu CY, Yeh YC, Shyr YM, Wu YY, et al. (2015) Erlotinib is effective in pancreatic cancer with epidermal growth factor receptor mutations: a randomized, open-label, prospective trial. Oncotarget 6: 18162-18173.
7. Tomaino B, Cappello P, Capello M, Fredolini C, Ponzetto A, et al. (2007) Autoantibody signature in human ductal pancreatic adenocarcinoma. J Proteome Res 6: 4025-4031.
8. Tomaino B, Cappello P, Capello M, Fredolini C, Sperduti I, et al. (2011) Circulating autoantibodies to phosphorylated α-enolase are a hallmark of pancreatic cancer. J Proteome Res 10: 105-112.
9. Capello M, Ferri-Borgogno S, Cappello P, Novelli F (2011) α-enolase: a promising therapeutic and diagnostic tumor target. FEBS J 278: 1064-1074.
10. Pancholi V (2001) Multifunctional alpha-enolase: its role in diseases. Cell Mol Life Sci 58: 902-920.
11. Souza CE, Rego JP, Lobo CH, Oliveira JT, Nogueira FC, et al. (2012) Proteomic analysis of the reproductive tract fluids from tropically-adapted Santa Ines rams. J Proteomics 75: 4436-4456.
12. Ceruti P, Principe M, Capello M, Cappello P, Novelli F (2013) Three are better than one: plasminogen receptors as cancer theranostic targets. Exp Hematol Oncol 2: 12.
13. Principe M, Ceruti P, Shih NY, Chattaragada MS, Rolla S, et al. (2015) Targeting of surface alpha-enolase inhibits the invasiveness of pancreatic cancer cells. Oncotarget 6: 11098-11113.
14. Hildenbrand R, Schaaf A, Dorn-Beineke A, Allgayer H, Sütterlin M, et al. (2009) Tumor stroma is the predominant uPA-, uPAR-, PAI-1-expressing tissue in human breast cancer: prognostic impact. Histol Histopathol 24: 869-877.
15. Ulisse S, Baldini E, Sorrenti S, D'Armiento M (2009) The urokinase plasminogen activator system: a target for anti-cancer therapy. Curr Cancer Drug Targets 9: 32-71.
16. Bauer TW, Liu W, Fan F, Camp ER, Yang A et al. (2005) Targeting of urokinase plasminogen activator receptor in human pancreatic carcinoma cells inhibits c-Met- and insulin-like growth factor-I receptor-mediated migration and invasion and orthotopic tumor growth in mice. Cancer Res 65: 7775-7781.
17. Lund LR, Ellis V, Rønne E, Pyke C, Danø K (1995) Transcriptional and post-transcriptional regulation of the receptor for urokinase-type plasminogen activator by cytokines and tumour promoters in the human lung carcinoma cell line A549. Biochem J 310 : 345-352.
18. Schnepp BC, Johnson PR (2014) Vector-Mediated In Vivo Antibody Expression. Microbiol Spectr 2: AID-0016-2014.
19. Schnepp BC, Johnson PR (2014) Adeno-associated virus delivery of broadly neutralizing antibodies. Curr Opin HIV AIDS 9: 250-256.
20. Cappello P, Tomaino B, Chiarle R, Ceruti P, Novarino A et al. (2009) An integrated humoral and cellular response is elicited in pancreatic cancer by alpha-enolase, a novel pancreatic ductal adenocarcinoma-associated antigen. Int J Cancer 125: 639-648.
21. Cappello P, Rolla S, Chiarle R, Principe M, Cavallo F, et al. (2013) Vaccination with ENO1 DNA prolongs survival of genetically engineered mice with pancreatic cancer. Gastroenterology 144: 1098-1106.
22. Cappello P, Tonoli E, Curto R, Giordano D, Giovarelli M, et al. (2015) Anti-a-enolase antibody limits the invasion of myeloid-derived suppressor cells and attenuates their restraining effector T cell response. OncoImmunology
23. Díaz-Ramos A, Roig-Borrellas A, García-Melero A, López-Alemany R (2012) α-Enolase, a multifunctional protein: its role on pathophysiological situations. J Biomed Biotechnol 2012: 156795.