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Hypotheses: A New Way Against Cancer Metastasis, Chitooligosaccharides as Mucosal Adjuvant for Therapeutic Vaccination Targeting Heparanase

Haizhi Quan and Zirong Xu
Key Laboratory for Molecular Animal Nutrition of Ministry of Education,
Institute of Feed Animal Sciences, Zijingang Campus, Zhejiang University, 310030 Hangzhou, China

Abstract: Heparanase is a tumor metastasis associated antigen so cancer vaccine targeting heparanase is an effective way to treat tumor metastasis. However, cancer vaccine needs adjuvant because of low immunostimulatory. Chitooligosaccharides (COS) has been proved to be an successful cancer vaccine adjuvant due to its propertied. COS will be a well adjuvant for cancer vaccine targeting heparanase.

Key words: Heparanase, chitooligosaccharide, tumor metastasis, adjuvant, cancer vaccine

INTRODUCTION

About 90% of cancer patient death is caused by tumor metastasis (Bogenrieder and Herlyn, 2003). Tumor metastasis is cancer cells escape from the primary tumor and seed at distant sites (Joyce and Pollard, 2009) Heparanase an endo-β-D-glucuronidase participates in degradation of the Extracellular Matrix (ECM) and removes the barriers between cells and tissues which lead to tumor cells migration and invasion. Heparanase is considered to be clinical relevance of the pro-metastatic (Ilan *et al*., 2006). To be attentive, the antibody, targeting heparanase is effective to inhibit tumor cells invasion (He *et al*., 2004).

Solid tumor and metastatic cells can be successfully eliminated by immunotherapy treating (Schreiber and Rowley, 2010). Therefore, most of researches of cancer immunotherapy are focused on cancer vaccines which are easily administrated and few side effects (Rosenberg *et al*., 2004). More and more tumor antigens have been recognized including associated metastasis (Wenandy *et al*., 2008). Heparanase has been characterized to be a metastasis correlating antigen which activates memory T lymphocytes in breast cancer patients (Sommerfeldt *et al*., 2006). And B cell epitopes of heparanase has been identified in hepatocellular carcinoma (Yang *et al*., 2009a). It is possible to develop cancer vaccine targeting heparanase. However, low immunostimulation caused by immune escape that tumor cells eradicate immune surveillance is the hindrances in cancer vaccine development (Prendergast, 2008). It is necessary to add adjuvant, the compound enhancing immune response in the cancer vaccine (Petrovsky and Aguilar, 2004). While cancer vaccine adjuvant should have the characteristics as follows: safety, highly immunostimulatory and anti-angiogenesis (Mesa and Fernandez, 2004), so its material needs high standards to have all of the qualities.

Mucosal immunization is an attractive vaccination, due to its safety, increasing both humoral and cellular immune response and needless needle administration (Couckea *et al*., 2009). Adding mucosal adjuvant in cancer vaccine will undoubtedly abate the pain of cancer patients, hence it is necessary to screen the material for the mucosal adjuvant of cancer vaccine. Chitosan, obtained from the deacetylated chitin sourced from crustacean shells is a non-toxic, biodegradable, immunostimulatory and mucosal permeable polysaccharide (Mao *et al*., 2010). Chitosan has the potential to be a cancer vaccine adjuvant because of its safety and well mucosal immunization (Illum *et al*., 2001). And chitosan has demonstrated synergistic therapeutic efficacy in mice pulmonary carcinoma cell xenograft (Han *et al*., 2008). But, the property that chitosan is only solubilized in acidic aqueous solution (Suh and Matthew, 2000) which leads to denature protein, limits the widespread application of chitosan (Karagozlu *et al*., 2010). Fortunately Chitooligosaccharides (COS), the oligosaccharides obtained by hydrolyzing chitosan (Xia *et al*., 2011), possesses the characteristics different from chitosan for example water solubility at the same time that it holds the properties similar to those of chitosan including safety (Wei *et al*., 2009). COS safely enhances the pulmonary absorption of interferon-α in rats (Yamada *et al*., 2005) and successfully improves the production of antibody induced by atherosclerosis DNA

Corresponding Author: Zirong Xu, Key Laboratory for Molecular Animal Nutrition of Ministry of Education,
Institute of Feed Animal Sciences, Zijingang Campus, Zhejiang University, 310030 Hangzhou, China

vaccine in rabbits after intranasal administration (Yang *et al*., 2009b). Moreover, after intraperitoneal injection or intranasal administration, COS based antisense oligodeoxynucleotide of interleukin-5 has shown treating allergic rhinitis effects in Murine Model (Kim and Kim, 2007). These animal experiments suggest that COS has all the elements of mucosal adjuvant for vaccine and can be formed into complex vaccine with the antigen in clinical application whatever this antigen is protein or nucleic acid. COS also protects the antigen from enzymolysis *in vivo*. COS will be the strong contender for the mucosal adjuvant of cancer vaccine due to its special characteristics such as immunostimulant effects (Kim and Rajapakse, 2005), anti-angiogenic activity (Wu *et al*., 2008), neutral water solubility (Artan *et al*., 2010) and mucosal permeability (Chae *et al*., 2005).

HYPOTHESES

At the premise of keeping the molecular structure of heparanase antigen, COS and heparanase antigen form into a complex vaccine due to the water solubility of COS. This complex vaccine will stimulate intensively mucosal immune response to treat tumor metastasis, via oral or intranasal administration, owing to the anti-angiogenesis activity, immunostimulatory and mucosal permeability of COS.

DISCUSSION

Haparanase is widely involved in tumor metastasis for example it plays an important role in melanoma cells migration by degrading its substrate heparan sulfate in ECM (Roy and Marchetti, 2009). Furthermore, heparanase expression is the poor prognostic indicator in pancreatic adenocarcinoma (Rohloff *et al*., 2002) and gastric cancer (Takaoka *et al*., 2003), respectively. Diagnosed heparanase positive expression is the symbol of metastatic tumor in cancer patients (Nadir and Brenner, 2009). So, it is urgent to develop cancer vaccine targeting heparanase. In normal tissue, the expression of human heparanase mRNA is limited in placenta and lymphoid organ while the heparanase mRNA level in malignancies or xenograft tumor is much higher than that of in corresponding normal tissue such as human breast, colon, lung, prostate, ovary and pancreas tumors comparing to corresponding normal tissue (Vlodavsky and Friedmann, 2001). The expression of heparanase is strictly regulated by tumor suppressor p53 in normal tissue (Baraz *et al*., 2006). In comparison, in solid tumor, tumor suppressor p53 is inactivated in the tumors cells which lost their apoptotic potential under the selective pressure mediated by hypoxia (Graeber *et al*., 1996). Since, the expression of heparanase is unlimited in metastatic tumor cells and the heparanase antigen is widespread in metastatic tumor. Cancer vaccine targeting heparanase will be safe and have therapeutic efficacy to the cancer patients suffering tumor metastasis.

The Cytotoxic T Lymphocytes (CTLs) epitopes of heparanase have been identified and the CTLs response activated by heparanase antigen not only lyses gastric tumor cells *in vitro* (Cai *et al*., 2007) but also inhibits pulmonary cells xenograft growth in mice (Tang *et al*., 2008). Cancer vaccine targeting heparanase will be practical in clinical application. However, cancer vaccine always needs adjuvant because of low immnostimulation. This problem will be solved by COS as cancer vaccine adjuvant. Firstly, COS has been proved to be an immune potentiator. COS activates murine macrophage and Dendritic Cells (DCs), via mannose (Han *et al*., 2005) and Toll-like receptor 4 receptor (Villiers *et al*., 2009), respectively.

Both macrophage and DCs belong to Antigen Presenting Cells (APCs) and APCs activate the effector cells of the immune defense which is the successful vaccination of vaccine antigen. Especially, DCs are considered to be professional APCs because DCs efficiently stimulate primary immune response and establish immunologic memory (O'Hagan and Valiante, 2003; Foged *et al*., 2002). After oral administration, COS significantly enhances T helper cell type 2 (Th2)/Th3 and natural killer cells activity in rats (Porporatto *et al*., 2005) and in mice (Maeda and Kimura, 2004), respectively. These demonstrated the immunostimulatory of COS, the first factor of adjuvant. The water solubility of COS keeps the immunogenecity of antigen because of not demolishing the molecular structure of antigen. Oral or intranasal administration is very valuable for diminishing patient pain because of needle free. Secondly, COS still has the safe characteristic of chitosan. Finally, COS has the anti-angiogenic activity which is the special requirement for cancer vaccine adjuvant. Angiogenesis is the process of growing new blood vessels from existed vessel and the process is induced by Vascular Endothelial Growth Factor (VEGF). Angiogenesis is very important to tumor metastasis for providing nourishment. High haparanase activity aggravates angiogenesis (Ilan *et al*., 2006). Tumor uses VEGF to impair DCs activity which leads to immunization failure (Mesa and Fernandez, 2004). The anti-angiogenic activity of COS solves it and anti-angiogenesis results in inhibiting tumor metastasis (Prashanth and Tharanathan, 2005). By the way, it should be explanation that COS may inhibit haparanase activity to suppress tumor metastasis (Quan *et al*., 2009) which will help heparanase vaccine to treat tumor metastasis.

CONCLUSION

Though, hsp110, the chaperone of heparanase, co-operating heparanase antigen has got anti-tumor therapeutical efficacy in mice (Hu *et al*., 2009). Chitin, the material of COS is one of the most natural polysaccharides in the world (Rinaudo, 2006), so the cost of producing COS will be lower and lower with the manufacturing progress which will benefit the cancer patients. In particular, the cancer patients will lessen pain by oral or intranasal administrated COS complex vaccine. In a word, the COS/heparanase antigen complex vaccine will treat tumor metastasis in many respects and COS will be a successful adjuvant after aluminium.

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