Immunomodulation by PSK Augments the Anti-tumor Effect of Paclitaxel in Mice with Locally Advanced Breast Cancers

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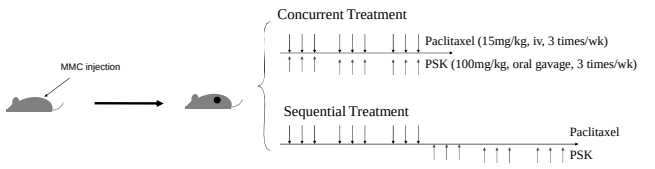
ABSTRACT

Locally advanced breast cancer (LABC) poses a significant clinical challenge because it is associated with a high relapse rate and poor overall survival. HER2+/ER- LABC is more aggressive and has been linked to poorer prognosis. The standard treatment for LABC patients is neoadjuvant therapy followed by surgery. Patients that achieved pathological complete response after neoadjuvant therapy had better overall suvival. We hypothesize that the addition of polysaccharide krestin (PSK), an immunomodulatory agent, to standard neoadjuvant therapy may augment the anti-tumor immunity and result in improved overall survival. To test this hypothesis, we used tumor-bearing neu transgenic mice as a model of HER2+/ER- LABC. Tumor-bearing mice received PSK during or after paclitaxel treatment. Results showed that concurrent PSK treatment or post-paclitaxel PSK treatment both potentiated the anti-tumor effect of paclitaxel. Preliminary mechanistic studies showed that PSK increased IL-12 secretion by dendritic cells and Type I cytokine secretion by T cells. Systemic PSK treatment increased tumor antigen-specific T cells in spleen and decreased the T regulatory cells in tumor. Data presented suggest that the inclusion of PSK into standard neoadjuvant therapy may improve tumor response.

BACKGROUND AND HYPOTHESIS

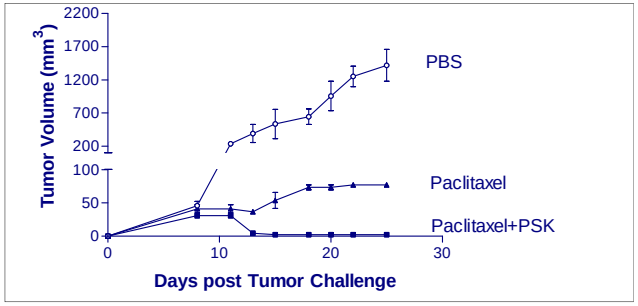
- **Locally Advanced Breast Cancer (LABC)** refers to large breast tumors (>5cm) or breast tumors involving skin and muscle, or with extensive involvement of regional LNs. The clinical management of LABC is challenging due to high relapse rate. Novel approaches are needed for this patient population.
- **Neoadjuvant (pre-operative) chemotherapy** followed by surgery is the standard treatment for LABC. Neoadjuvant chemotherapy can reduce tumor size making the disease more amenable to surgery. In approximately 30% of patients, biopsy at surgery can demonstrate a pathologic complete response (PCR), which has been associated with improved long-term survival. Novel approaches that may improve the efficacy of neoadjuvant therapy resulting in a greater incidence of PCR will greatly benefit breast cancer patients.
- **Neoadjuvant therapies which incorporate immune based treatments may be more effective than standard therapies alone.** The use of chemotherapy can have potent immunomodulatory effects (Zitvogel, et al, Nat Rev Immunol, 2008). Chemotherapy can result in release of tumor antigens. The addition of immune based therapy to chemotherapy may result in enhanced antigen presentation and augment the function of T cells.
- **Polysaccharide Krestin (PSK) is a mushroom extract that has immune modulatory effect.** PSK is a high MW polysaccharide (95-100kDa) isolated from *Coriolus Versicolor* Mushroom *(Yunzhi)*. PSK has long been used in Asia for its immune stimulatory and anti-tumor effect. PSK also inhibits tumor growth in neu trangenic mice. This natural immunomodulatory compound may be an addition to standard neoadjuvant therapy.
- **HYPOTHESIS: The immunomodulatory effect of PSK may synergize with the tumoricidal effect of paclitaxel and result in augmented anti-tumor response.**
- **Neu transgenic mice as a model of human breast cancer**
  The tumors are immunogenic and the tumor antigen repertoire is similar to that in human. (Lu et al, Can Res, 2006)
  The anti-tumor immunity is dampened by the high levels of suppresive T regulatory cells and removal of Tregs can augment anti-tumor immunity. (Knutson et al, JI, 2006)
- **The long term goal is to develop novel neoadjuvant therapy that combines chemo- and immunotherapy for patients with LABC.**

METHODS

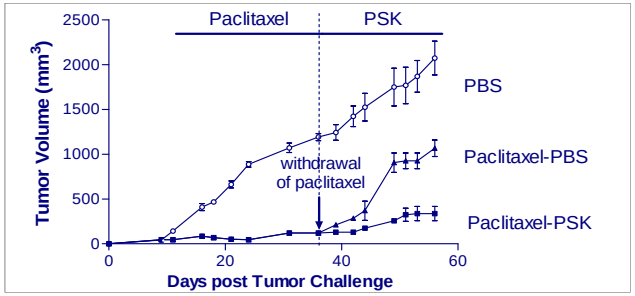


RESULTS

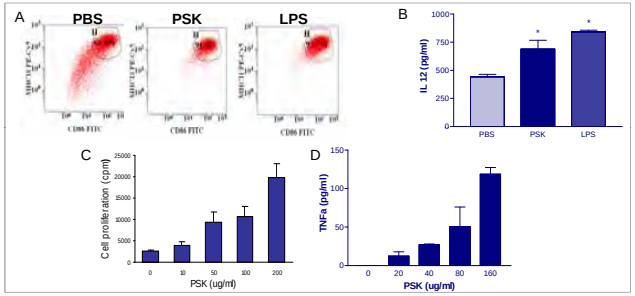
1. The addition of PSK potentiated the anti-tumor effect of paclitaxel. Tumor-bearing mice received paclitaxel, with or without concurrent PSK for 2 weeks. At the end of the treatment, the tumor size in the combination treatment group ($3.1\pm3mm^3$) is significantly smaller than that in paclitaxel alone group ($76.9\pm0mm^3$, p=0.002).



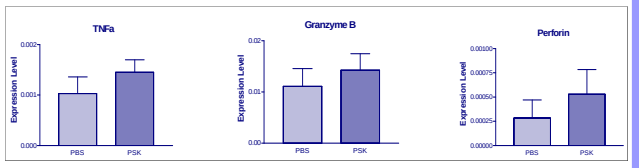
2. PSK administerd after paclitaxel inhibited tumor regrowth after paclitaxel withdrawal. Tumor-bearing mice received 3 weeks of paclitaxel or control PBS treatment. After paclitaxel withdrawal, half of the mice that were in paclitaxel group received PSK (100mg/kg, oral gavage, 3 times a week) and the other half of the mice were left untreated. Tumor growth was followed for another 3 weeks. On day 56, the tumor size in mice that received PSK after paclitaxel was $336\pm79mm^3$, significantly smaller than tumor size in mice that did not receive PSK after paclitaxel ($1065\pm92mm^3$, p=0.03).



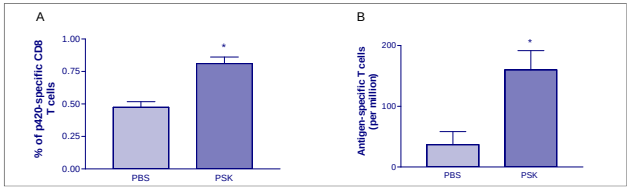
3. PSK induced IL-12 secretion by DC and Type I cytokine secretion by T cells. (A) PSK treatment on bone marrow-derived DC increased the level of mature DC (CD86+MHCIIhi). (B) PSK treatment on bone marrow-derived DC resulted in increased secretion of IL-12 by DC. (C) PSK (96h) stimulated the proliferation of splenocytes from neu-tg mice dose-dependently. (D) PSK (96h) stimulated the secretion of TNFalpha by splenocytes dose-dependently.



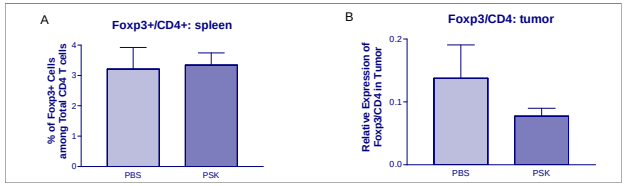
4. PSK up-regulated the expression of immune response-related genes in the tumor after systemic treatment. Shown are the relative expression levels (mean±sem) of TNF-α, granzyme B, and perforin in tumors from PSK and control PBS-treated mice. The mRNA expression levels of each gene was measured by real time RT-PCR and normalized to beta-actin.



5. PSK augmented anti-tumor immunity in neu transgenic mice. After 3 weeks PSK treatment, the antigen-specific T cells increased in spleen. (A) FACS data showing that the percentage of rat neu (p420)-specific T cells among total CD8 T cells increased from 0.47±0.04% to 0.81±0.05% (*, p<0.05). (B) ELISPOT data showing that the tumor antigen-specific precursors increased from 36.7±21.7 to 160.0±31.7 precursors per million T cells (*, p<0.05).



6. PSK treatment decreased the level of T regulatory cells in tumor but not in spleen. (A) The percentage of Foxp3+ cells among total CD4+ T cells in spleen as measured by flow cytometry. Shown are the mean±sem (n=5) in each treatment group. (B) The mRNA expression ratio of Foxp3/CD4 in tumors from control and PSK-treated tumors (n=3 in PBS, n=5 in PSK group).



CONCLUSIONS

- PSK, when used concurrently with paclitaxel, can potentiate the anti-tumor effect of paclitaxel.
- PSK, when used post-paclitaxel treatment, can inhibit tumor regrowth after paclitaxel withdrawal.
- PSK augmented tumor-specific immune response, possibly through multiple mechanisms, including decreasing the level of T regulatory cells in tumor, up-regulating the immune response-related genes in tumor, and augmenting the function of DC and T cells.

ACKNOWLEDGEMENTS

This work was supported by NIH grant RO1 AT004314-01.

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