Naturally Occurring Nanoparticles from Fungi for Tumor Immunochemotherapy

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Statement of Purpose: A carnivorous fungus *Arthrobotrys oligospora* has been shown to secrete fungal nanoparticles (FNPs) in a sitting drop culture system [1]. In the present work, the potentials of the FNPs in cancer therapy as a multifunctional nanocarrier were explored *in vitro* by investigating immunostimulatory activities, mechanisms of the cytotoxicity, and immunochemotherapeutic effects.

Methods: *Arthrobotrys oligospora* (ATCC 24927) was cultured in the sitting drop culture system proposed early [1] with minor modification. A new isolation procedure, size exclusive chromatography (SEC) - weak anion exchanger (WAX) -SEC, was established to purify the FNPs. Atom force microscopy (AFM) and dynamic/ electrophoretic light scattering (DLS/ELS) were used to characterize the FNPs. The immunostimulatory activity of the FNPs was determined in mouse macrophage RAW 264.7 cells and splenocytes derived from C57BL/6 mouse. MTT assay, TUNEL assay and propidium iodide staining were used for the cytotoxicity of the FNPs, apoptotic effect and cell cycle arrest in the tumor cells. A co-culture system where B16BL6 tumor cells and the splenocytes were co-cultured was used to evaluate the immunotherapeutic effect of the DOX-FNP complexes.

Results:

To prepare the FNPs with diameters less than 200 nm, a new isolation procedure, SEC-WAX-SEC, was established. Two FNP fractions (FNP1 and FNP2) were obtained from the crude FNPs using the procedure (**Fig.1 A**). AFM imaging revealed a reduced diameter of 100-200 nm for the two purified FNPs (**Fig. 1B-C**), as compared with the crude FNPs reported early [1]. The FNP1, with zeta potential of ~ -27 mV, was lower than that of FNP2 (~ -32mV). SDS-PAGE analysis revealed that polysaccharides, including acidic glycosaminoglycan and neutral polysaccharides, were the main chemical components in the FNPs. The purified FNPs enhanced the secretion of multiple proinflammatory cytokines (IL6, TNF-α, G-CSF, IL-1α, IL-1β, and IL2) and chemokines (TRANTES, MCP-1, IP-10, MDC, MIP-1a, MIP-1b, TARC, KC, and NO) from macrophages and splenocytes (**Fig. 2**). MTT assay showed that the two purified FNPs had mild cytotoxicity against multiple tumor cells (**Fig. 2**), but the FNP2 had stronger cytotoxic activity than the FNP1. The differences in the cytotoxic activity between the two FNPs were substantiated by apoptosis and cell cycle analysis. It is the FNP2 not the FNP1 that could clearly inhibit cell proliferation via inducing apoptosis and arresting tumor cells at sub G0/G1 phase. Both FNPs can form the pH-responsive nanocomplexes with doxorubicin (DOX) via electrostatic interactions. In a direct cytotoxicity experiment, the DOX-FNP2 complexes demonstrated higher cytotoxic activity than the free DOX against multiple tumor cells, while the cytotoxic activity of the DOX-FNP1 complexes was weaker than the free DOX. Interestingly, in a co-culture experiment where splenocytes were co-cultured with tumor cells, both DOX-FNP complexes demonstrated higher antitumor activity than the free DOX, suggesting a synergistic effect between the immunostimulation of the FNPs and cytotoxicity of the nanocomplexes *in vitro*.

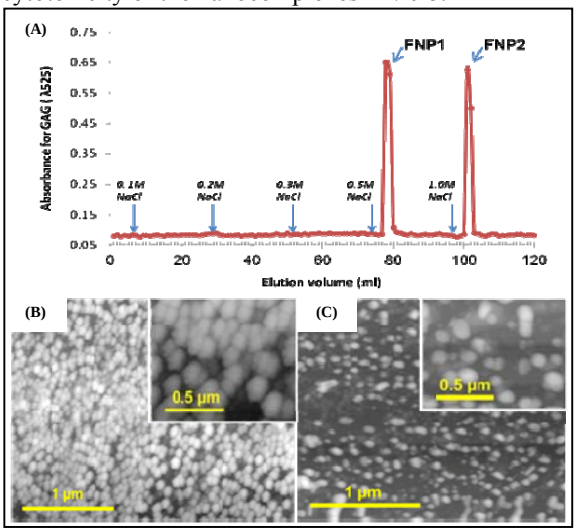

Figure 1. Two FNPs fractions were purified from the culture media (A), with a reduced diameter of 100-200 nm for the FNP1 (B) and the FNP2 (C).

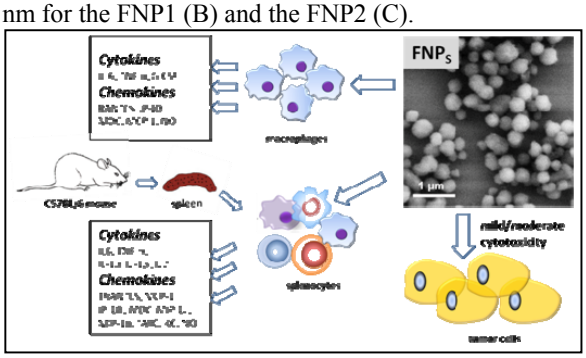

Figure 2. The FNPs induced secretion of multiple proinflammatory cytokines and chemokines from *in vitro* cultured macrophages RAW264.7 and primary splenocytes derived from C57BL/6 mouse.

Conclusions: We have developed a one-step therapy containing agents for both immuno- and chemo-therapy using the fungal nanoparticles as a multifunctional nanocarrier, which may open a new avenue for combined cancer therapy.

References: [1] Wang Y., et al, Adv Funct Mater. 2013;23:2175-2184.