Single molecule analysis of toll-like receptor and related cancer immunotherapy

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Goals:

- To investigate the binding model of TLR's ligand and TLR in cancer and immune cells by Total internal reflection fluorescence.
- To validate the TLR intracellular colocalization by FCS and FLIM.
- To develop a multifunctional nanoparticles system loaded with CpG DNA for breast cancer immunotherapy

Statement of Problem: The mammalian immune system is comprised of two branches: innate and acquired immunity. Toll-like receptors (TLRs) are the key sensors of microbial infection in mammals. They lie at the core of our inherited resistance to disease, inducing the modulation of hundreds of host genes. Almost all phenomena associated with infection are traceable to TLRs. Much interest has focused on TLR4 because of its ability to respond to Lipopolysaccharides (LPS). It is believed that the association of the MD-2:LPS complex could greatly enhance the sensing of LPS. However, additional evidence challenges this attractive hypothesis that surface expression of TLR4 is regulated by MD-2 coexpression. The "helper" role of MD-2 appeared to be restricted to the mouse model. Hence, we propose to use total internal reflectance fluorescence microscopy to investigate the binding mode of LPS/TLR4. TLR9 recognizes the unmethylated CpG nucleotides. But it is a big challenge to activate DC in cancer patients in cancer immunotherapy because DC at the tumor site are often dysfunctional and unable to prime T cells efficiently. CpG, which could activate DC by stimulating TLR9, through the multiple mechanisms of a direct antitumor effect, an enhancement of antigen (Ag) presentation function by the tumor and synergy with cytotoxic agents to induce a T cell immune response against the tumor. Multifunctional nanoparticle systems could delivery CpG DNA by injection from the caudal vein of mice efficiently to trigger the innate and adaptive immune systems cells both in the host and in the tumor microenvironment itself.

Current Activities: Ligand (LPS) are labeled by Alex Fluorescence. Binding mode of Ligand and corresponded receptor will be done by Total internal reflection fluorescence microscopy (TIRFM) in different cell lines to observe the binding details of the TLR4-MD2/LPS complexes. Fluorescence Life Time Imaging (FLIM) and Fluorescence Correlation Spectroscopy (FCS) will be used to investigate the colocalization and ligand recognization of TLR9, TLR8 and TLR3. An Animal model bearing breast cancer will be established. Multifunction nanoparticles loaded CpG DNA that could efficiently initiate innate and adaptive immune response combined with chemical therapy drug will be conducted for the cancer immunotherapy.