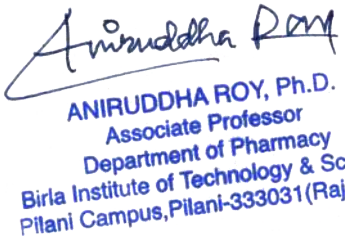
Summary

Title: Development of pH-Sensitive Nanoparticles for the Combined Chemo-Immunotherapy of Cancer

Recent advanced studies have underscored cancer's multidimensional nature. However, most cancer therapies remain one-dimensional, targeting only a single facet of the intricate tumor ecosystem. Unfortunately, such singular approaches often prompt the TME to adopt a more aggressive nature, fueling resistance. To enhance therapeutic outcomes, embracing a multidimensional strategy becomes imperative. One such emerging promising avenue is the concept of combined chemo-immunotherapy. However, many of the chemotherapeutic agents exhibit inherent immunosuppressive traits, rendering them unsuitable for this approach. Exceptions like paclitaxel (PTX) stand out, displaying immune-stimulating properties at therapeutic levels with improved cross-priming of antitumor CD8+ T cells and enhanced immune cell infiltration. However, effective T-cell activation hinges on antigen presentation. Macrophages, the primary antigen-presenting cells in TME, often exist in an immune-suppressed state. Toll-like receptor (TLR) agonists exhibit potent immunogenic properties by triggering macrophage activation. Consequently, the targeted delivery of both chemotherapeutic agents and TLR agonists to tumors holds significant potential for synergistic chemo-immunotherapy. Given their distinct cellular targets, maintaining the free form of these agents within the TME is vital. A stimuli-responsive NP delivery system could shield these agents during systemic circulation and facilitate their controlled release within the TME, effectively engaging their specific target cells.

In the current study, initial screening of different TLR-agonists was performed. The most efficacious one, resiquimod (RSQ), was selected to be combined with PTX. For tumor-targeted delivery of this drug combination, we have synthesized Poly-histidine-conjugated star-shaped PLGA and linear PLGA as a pH-sensitive polymer. The microfluidic-based nanoprecipitation method yielded uniform-sized NPs, exhibiting a pH-dependent drug release profile. A comparative study was done between two different pH-sensitive nano-delivery systems, with a linear polymer and multi-armed, star-shaped polymer, to evaluate the impact of polymer structure on the pH-sensitive property. The NPs prepared with the multi-arm polymer (spH-NP) exhibited improved efficacy in terms of pH-dependent size variation and drug release, as well as spheroid penetration. They led to an enhanced cancer cell death through increased macrophage activation in an *in-vitro* complex breast cancer spheroid model (breast cancer cell + macrophage cell). The superior pH-sensitive property of the spH-NP resulted in enhanced combined chemo-immunotherapeutic activity with improved cancer cell death, reduced macrophage cell death, and M2 to M1 conversion. Further, *in-vivo* evaluation confirmed safety across all the treatment groups. The pharmacokinetic profile showed improved PTX and RSQ plasma circulation with spH NPs. *In-vivo*, PTX+RSQ outperformed PTX and RSQ alone in antitumor efficacy. spH-NPs exhibited the highest antitumor activity, inducing metastasis inhibition.

Overall, the current study suggests that the combination of PTX and RSQ has potent chemo-immunotherapeutic activity. A stimuli-responsive carrier system with a payload deployed for targeting TME and cancer cells markedly improved its *in-vitro* 2D, 3D, as well as *in-vivo* efficacy. Thus, this study opens a new door for developing stimuli-responsive smart NPs for multidimensional tumor therapy.



Nominator/Supervisor

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