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論　文　内　容　要　旨

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学位論文題目	The potential effect of iontophoresis technology on the delivery of various melanoma vaccines into the skin （イオントフォレシス技術を用いた各種メラノーマワクチンの皮内送達による抗がん効果）

Although the strategy in cancer vaccination is to provide a therapeutic effect against an established tumor, there is an urgent need to develop prophylactic vaccines for non-viral cancers. Polyplex nanoparticles were prepared through electrostatic interactions between a positively-charged modified tumor associated antigen, namely human derived melanoma $gp100_{25\text{-}33}$ peptide (KVPRNQDWL-RRRR), and a negatively charged cytosine-phosphate-guanosine motif (CpG-ODN) adjuvant. We previously demonstrated successful transdermal delivery of various hydrophilic macromolecules by iontophoresis (IP) using weak electricity. Herein, I investigated the effectiveness of IP in the transdermal delivery of a prophylactic polyplex vaccine. IP was successful in establishing a homogenous distribution of the vaccine throughout skin. Efficacy of the prophylactic vaccine delivered either by IP or subcutaneous (s.c) injection was demonstrated against melanoma growth. A significant tumor regression was observed, which was confirmed by elevated mRNA expression levels of various cytokines, mainly interferon (IFN)-γ, as well as infiltration of cytotoxic $CD8^+$ and $CD4^+$ T cells in the tumor tissue. Additionally, I evaluated the therapeutic effect of the vaccine and I found a potent and a significant reduction in tumor burden. Also, stimulation of systemic immunity was confirmed by upregulation of serum IFN-γ levels. Finally, results of both prophylactic and therapeutic studies showed a non-significant difference between IP and s.c injection.

Unlike DNA vaccines, mRNA vaccines lack the possibility for genomic integration, resulting in minimal concerns for gene disruption, mutagenesis and tumorigenesis. Moreover, mRNA vaccines are distinguished by their short half-lives and well-tolerated safety profile, as well as their rapid, high-yield, safe, and cost-effective production. Additionally, mRNA vaccines have attracted considerable attention as a result of the 2019 coronavirus pandemic; however, challenges remain regarding use of mRNA vaccines, including insufficient delivery owing to the high molecular weights and high negative charges associated with mRNA. These characteristics of mRNA vaccines impair intracellular uptake and subsequent protein translation. Therefore, a minimal mRNA vaccine (M.W:20,460) encoding a tumor associated antigen human $gp100_{25\text{-}33}$ peptide (KVPRNQDWL), as a potential treatment for melanoma was chemically synthesized. Minimal mRNA which includes short poly (A) tail has recently shown promise at improving the translational process, and can be prepared via a simple production method. Moreover, my laboratory previously reported the successful use of IP technology in the intracellular delivery of siRNA. I hypothesized that combining IP technology with a chemically synthesized minimal mRNA vaccine can improve both transdermal and intracellular delivery of minimal mRNA vaccine. Therapeutic minimal mRNA vaccine was administered either by IP or s.c injection. Following IP-delivered minimal mRNA vaccine, an immune response is elicited resulting in activation of skin resident immune cells. As expected, combining both technologies led to potent stimulation of the immune system, which was observed via potent tumor inhibition in mice bearing melanoma. Additionally, there was an elevation in mRNA expression levels of various cytokines, mainly IFN-γ, as well as infiltration of cytotoxic $CD8^+$ and $CD4^+$ T cells in the tumor tissue, which are responsible for tumor clearance. Also, stimulation of systemic immunity was confirmed by upregulation of serum IFN-γ levels. Finally, minimal mRNA vaccine showed a Potent and higher effect after using IP technology compared to the s.c injection. This is the first report which combines IP technology with a chemically synthesized minimal mRNA vaccine.