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Development of vaccine against human papillomavirus types 3, 10 and 77

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Human Papillomaviruses HPVs comprise a large family of viruses known as causative agents for proliferative epithelial lesions in man. The so called high-risk HPV types (e.g. HPV16 and HPV18) are causative agents of cervical cancer and prophylactic vaccines against both of these cancer-related HPV types, HPV16 and HPV18, have been produced based on recombinant expression of the major capsid protein L1. The cutaneous HPV types induce benign skin lesions. However, in immunosuppressed patients, e.g. organ transplant recipients (OTRs), skin lesions related to HPV are common and recalcitrant to treatments. Cutaneous HPVs including HPV3, 10 and 77 are the predominant HPV types detected in skin lesions of OTRs. Pre-operative vaccination may be a way to protect OTRs from cutaneous HPV infection during post-operative immunosuppressive therapy.

The aim of this thesis was to establish efficient and optimized production and purification protocols for recombinant L1 proteins (capsomeres and virus-like particles, VLPs) of HPV3, 10 and 77 which are vaccine candidates to prevent cutaneous HPV infection in OTRs. For this purpose, L1 proteins of HPV3, 10 and 77 were expressed in and purified from *E. coli* and insect cells. The L1 proteins were characterized structurally (electron microscopy, size exclusion gel filtration and sedimentation analysis) and immunologically. L1 proteins expressed in *E. coli* assembled into capsomeres while L1 proteins expressed in insect cells assembled into VLPs. Moreover,

capsomeres produced in *E. coli* were capable of further assembling *in vitro* into VLPs. The structures of *in vitro*. assembled VLPs resembled those of VLPs produced from insect cells. Immunization of mice with L1 capsomeres elicited L1-specific antibody response. The L1-specific antibodies showed cross reactivity not only to the same HPV species (e.g. alpha 2: HPV3, 10 and 77), but also to different HPV species (e.g. alpha 9: HPV16) and genera (e.g. gamma HPV: HPV4). Type-specific and cross-reactive monoclonal antibodies were generated from mice immunized with the recombinant L1 proteins of HPV 3, 10 and 77.

The recombinant L1 proteins of HPV3, 10 and 77 in the forms of capsomeres and VLPs which were produced, purified and analyzed in this are potential candidates for prophylactic vaccines. In particular, the *in vitro*. VLP assembly from bacterially expressed capsomeres provides a way to produce low-cost VLP vaccines especially for the developing countries.