UNIVERSITE CLAUDE BERNARD – LYON I

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Titre de la thèse : « Les oncoprotéines E6 et E7 du papillome humain de type 38 modifient la réponse cellulaire induite par les UV en inhibant l'expression du récepteur Toll-like 9. »

Résumé de la thèse

The human papillomaviruses (HPV) consist of a group of capsid-enclosed dsDNA viruses from the *Papillomaviridae* family that display a distinct tropism for mucosal or cutaneous squamous epithelia. Until now, more than 200 types of HPV have been isolated and grouped into a phylogenetic tree composed of 5 genera (alpha, beta, gamma, mu and nu papillomaviruses). Among them, the mucosal high-risk HPV types that belong to the genus alpha have been associated with cervical cancer as well as a subset of ano-genital and head and neck carcinomas. They are responsible for approximately 5% of all virus-induced cancers.

Beta HPV types have a skin tropism and have been suggested to be involved, together with ultraviolet light (UV), in the development of non-melanoma skin cancer (NMSC). For instance, *in vitro* and *in vivo* experimental models highlight the transforming properties of beta HPV38 E6 and E7. Specifically, studies of transgenic mouse model, where HPV38 E6 and E7 are expressed in the undifferentiated basal layer of epithelia under the control of the Keratin 14 (K14) promoter, showed a very high susceptibility to UV-induced skin carcinogenesis in comparison to the wild-type animals.

Equally important as their ability to promote cellular transformation, oncogenic viruses have different strategies to overtake the host immune-system thus guaranteeing persistent infection. Therefore, understanding whether potential oncogenic viruses have the ability to interfere with the immune response could provide additional evidence relating to their involvement in human carcinogenesis.

Here, we show that the E6 and E7 oncoproteins from HPV38 suppress the expression of the double-stranded DNA innate immune sensor TLR9 by promoting the accumulation of ΔNp73α, an antagonist of p53 and p73. Chromatin immunoprecipitation experiments show that ΔNp73α is part of a negative transcriptional regulatory complex that binds to a NF-κB responsive element within

the TLR9 promoter. Interestingly, ectopic expression of TLR9 in HPV38 E6E7 cells results in an accumulation of the cell cycle inhibitors $p21^{WAF1}$ and $p27^{Kip1}$, reduction of CDK2-associated kinase activity and inhibition of cellular proliferation. Together these data indicate that TLR9 is involved in additional events, besides the innate immune response. Accordingly, we observe that the treatment of human primary keratinocytes (HPK) with different cellular stresses, e.g. UV irradiation, doxorubicin and H_2O_2 treatment, results in TLR9 up-regulation. The UV-induced event is mediated by the recruitment of several transcription factors to the TLR9 promoter, such as p53, NF-kB p65 and c-Jun. The expression of HPV38 E6 and E7 strongly affect the recruitment of these transcription factors to the TLR9 promoter, with consequent impairment of TLR9 gene expression.

In summary, our data show that HPV38, similarly to other viruses with well-known oncogenic activity, can down-regulate TLR9. Most importantly, we highlight a novel function of TLR9 in controlling the cellular response to stresses and we show that HPV38 E6 and E7 are able to interfere with such mechanism. These findings further support the role of beta HPV types in skin carcinogenesis, providing additional insight into their precise contribution to the multi-step process of cancer development.