

Immunotherapy of HPV-associated head and neck cancer

Critical parameters

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Various arguments support the development of a vaccine targeting human papillomavirus (HPV) for the treatment of HPV-associated head and neck cancer. However, the mucosal localization of this tumor, the HPV-driven downregulation of MHC Class I molecules and various other immunosuppressive mechanisms must be carefully considered to improve the clinical efficacy of such an immunotherapeutic strategy.

Introduction

Oncogenic human papillomavirus (HPV) variants, mainly Type 16 HPV (HPV-16), have been robustly associated with head and neck cancer, in particular oropharyngeal cancer (OC). The proportion of OC cases caused by HPV ranges between 40 and 80%, depending on the geographical region. Although HPV⁺ lesions generally have a better prognosis than non-HPV-associated tumors, patients are treated with high-dose chemoradiotherapeutic regimens irrespective of their HPV status. In this context, HPV-targeting immunotherapy represents a complementary approach that may allow clinicians to employ conventional therapies at reduced doses, avoiding unwarranted toxicities. HPV-encoded proteins such as E6 and E7 are considered to be good targets for immunotherapy as (1) they are strictly required for the immortalization of keratinocytes and the continuous growth of the tumor, implying that they cannot be downregulated as a mechanism to escape immune attacks; (2) they are immunogenic in humans, both naturally and upon vaccination, eliciting specific T-cell and

humoral responses; (3) sporadic regressions of HPV-associated pre-neoplastic lesions have been observed in clinical trials testing therapeutic anti-HPV vaccines composed of E6- and E7-derived long peptides. These arguments strongly support the development of HPV-targeting vaccines for the treatment of HPV-associated head and neck cancer. However, some critical parameters must be carefully considered for the design and clinical application of these vaccines.

The Intranasal Route of Immunization is Required for Anticancer Vaccines to Induce the Regression of Head and Neck Cancer Lesions

In a recent study, we set up an orthotopic murine model of head and neck cancer expressing the HPV-16 proteins E6 and E7. Using a vaccine composed of the Shiga toxin B subunit, a vector targeting dendritic cells, coupled to an E7-derived long peptide, we found that the intranasal, but not the intramuscular, route of immunization is effective to cure established orthotopic head and neck tumors.¹ The

intranasal (mucosal) immunization also led to a more robust tumor infiltration by anti-E7 CD8⁺ T cells than the intramuscular route. Finally, the intranasal administration of the anti-HPV vaccine stimulated the expression of mucosal integrins (CD49a, CD103) on CD8⁺ T cells. Blockade of CD49a decreased both CD8⁺ T-cell infiltration and the therapeutic efficacy of the vaccine. This work identifies a link between the immunization route and the induction of a mucosal homing program on CD8⁺ T cells with a direct impact on the efficacy of anticancer vaccines for the treatment of head and neck cancers.¹ In particular, these results strongly suggest that the intranasal route of immunization should be preferred for the development of a therapeutic HPV-targeting vaccine against head and neck cancer.

Counteracting the Role of Anergic PD1⁺ T cells and Regulatory T Cells in the Local Microenvironment of Head and Neck Cancers

We and others have shown that head and neck cancer lesions generate a microenvironment that is characterized by high

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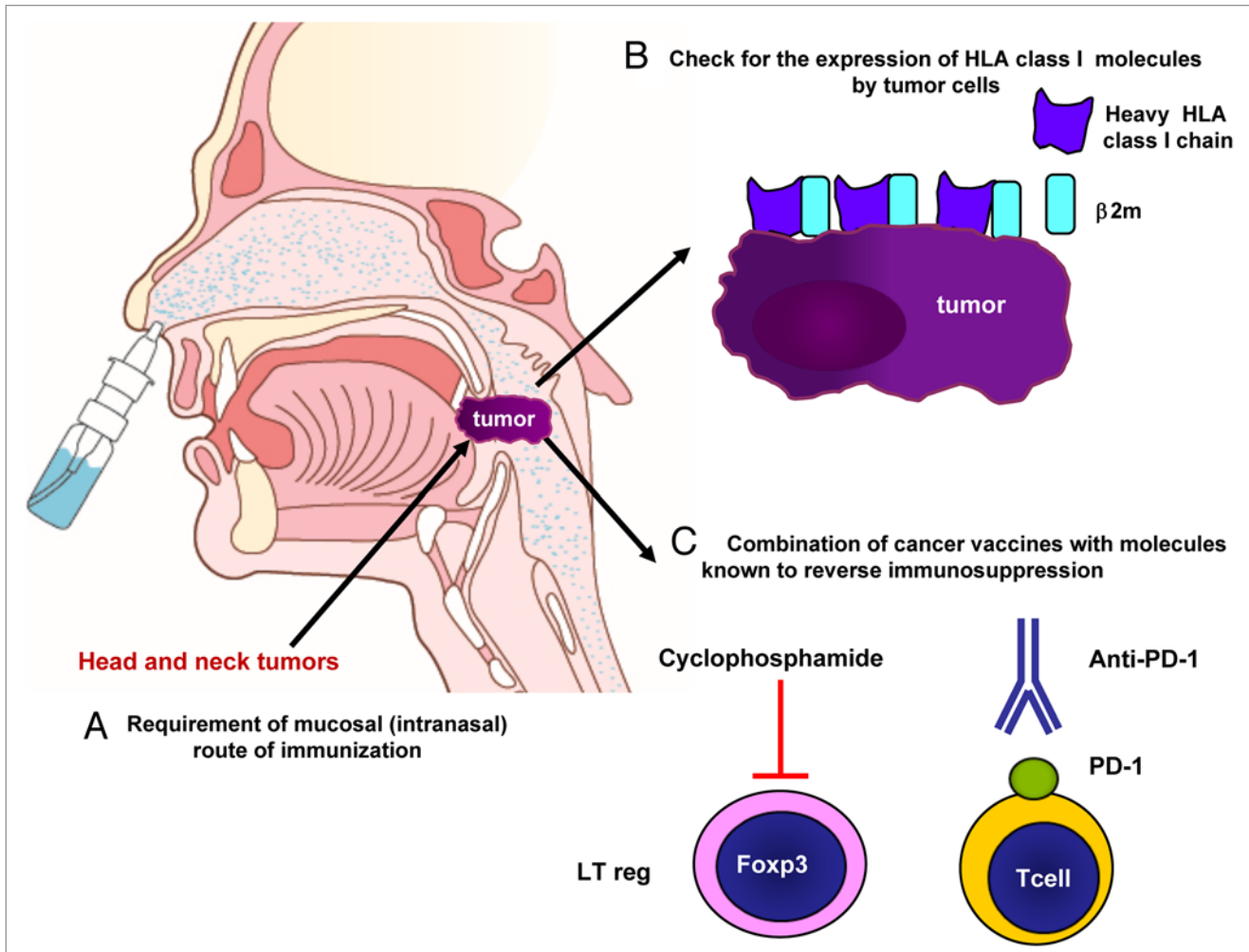


Figure 1. Critical parameters to improve the efficacy of therapeutic HPV-targeting vaccines in head and neck cancer patients. (A) The intranasal (mucosal) route of immunization significantly potentiates the efficacy of HPV-targeting vaccines. (B) As HPV proteins including E5 and E7 can downregulate MHC Class I molecules, measuring the expression of the latter on the surface of tumor cells may allow for the selection of patients who are most likely to respond to anticancer immune responses elicited by HPV-targeting vaccines. (C) Malignant cells activate various immunosuppressive mechanisms, including the recruitment of regulatory T cells (Tregs) and myeloid-derived suppressive cells, the activation of immune checkpoints on activated T cells, etc. Hence, drugs that alleviate immunosuppression should be combined with anticancer vaccines to improve their therapeutic potential.

levels of pro-inflammatory cytokines and robust tumor infiltration by immunosuppressive T cells including regulatory T cells (Tregs), immature myeloid cells and anergic PD1⁺ T cells.²⁻⁴ Although such an infiltration by immunosuppressive and anergic T cells has paradoxically been associated with good prognosis, these cells appear to maintain their inhibitory functions or anergic state.^{5,6} In pre-clinical models of head and neck cancer expressing HPV proteins, combining a HPV-targeting vaccine with the blockade of Tregs or the PD1-PD-L1 interaction improved the induction of anti-E7 CD8⁺ T cells and the regression of established tumors.³ In patients affected by cervical

carcinoma, which is also associated with specific HPV variants, the presence of Tregs has been associated with resistance to an anti-HPV vaccine,⁷ supporting these previous preclinical results. A cancer vaccine has been shown to synergize with the blockade of Tregs in renal cancer patients, highlighting the clinical potential of administering anticancer vaccines together with drugs that limit immunosuppression.⁸

MHC Status of HPV-Associated Head and Neck Cancer

The ultimate goal of HPV-targeting vaccines is to induce cytotoxic CD8⁺

T lymphocytes that eradicate HPV-associated head and neck cancer cells. In this scenario, it must not be forgotten that about 30% of HPV⁺ head and neck tumors do not express MHC Class I molecules,⁹ presumably as a consequence of the expression of the viral proteins E5 and E7. Indeed, E5 has been shown to retain the heavy chain of MHC Class I molecules in the endoplasmic reticulum, whereas E7 is known for its capacity to repress transcription from the MHC Class I genetic locus. Although they are not specific for HPV⁺ lesions, other mechanisms can lead to the downregulation of MHC Class I molecules by head and neck tumors, including the production of high levels of

gangliosides or SHP2 by malignant cells as well as abnormalities in their antigen-processing machinery.¹⁰

Conclusions

The development of HPV-targeting vaccines for the therapy of oropharyngeal tumors is a logical strategy based on to

the elevated incidence of HPV⁺ lesions as well as on promising preclinical and clinical results. However, the anatomical localization of head and neck cancers and their highly immunosuppressive micro-environment require these vaccines to be administered via the mucosal route and to be combined with strategies that limit immunosuppression (Fig. 1). Lastly, the

selection of patients whose tumors express MHC Class I molecules in spite of the presence of HPV can further enhance the clinical efficacy of this immunotherapeutic regimen.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest have been disclosed.

References

1. Sandoval F, Terme M, Nizard M, Badoual C, Bureau ME, Freyburger L, et al. Mucosal imprinting of vaccine-induced CD8⁺ T cells is crucial to inhibit the growth of mucosal tumors. *Sci Transl Med* 2013; 5:72ra20; PMID:23408053; <http://dx.doi.org/10.1126/scitranslmed.3004888>
2. Badoual C, Sandoval F, Pere H, Hans S, Gey A, Merillon N, et al. Better understanding tumor-host interaction in head and neck cancer to improve the design and development of immunotherapeutic strategies. *Head Neck* 2010; 32:946-58; PMID:20191626
3. Badoual C, Hans S, Merillon N, Van Ryswick C, Ravel P, Benhamouda N, et al. PD-1-expressing tumor-infiltrating T cells are a favorable prognostic biomarker in HPV-associated head and neck cancer. *Cancer Res* 2013; 73:128-38; PMID:23135914; <http://dx.doi.org/10.1158/0008-5472.CAN-12-2606>
4. Badoual C, Bouchaud G, Aguezny NelH, Mortier E, Hans S, Gey A, et al. The soluble alpha chain of interleukin-15 receptor: a proinflammatory molecule associated with tumor progression in head and neck cancer. *Cancer Res* 2008; 68:3907-14; PMID:18483276; <http://dx.doi.org/10.1158/0008-5472.CAN-07-6842>
5. Badoual C, Hans S, Rodriguez J, Peyrard S, Klein C, Aguezny NelH, et al. Prognostic value of tumor-infiltrating CD4⁺ T-cell subpopulations in head and neck cancers. *Clin Cancer Res* 2006; 12:465-72; PMID:16428488; <http://dx.doi.org/10.1158/1078-0432.CCR-05-1886>
6. Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a Role of the PD-1:PD-L1 Pathway in Immune Resistance of HPV-Associated Head and Neck Squamous Cell Carcinoma. *Cancer Res* 2013; 73:1733-41; PMID:23288508; <http://dx.doi.org/10.1158/0008-5472.CAN-12-2384>
7. Welters MJ, Kenter GG, de Vos van Steenwijk PJ, Löwik MJ, Berends-van der Meer DM, Essahsah F, et al. Success or failure of vaccination for HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses. *Proc Natl Acad Sci U S A* 2010; 107:11895-9; PMID:20547850; <http://dx.doi.org/10.1073/pnas.1006500107>
8. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multi-peptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat Med* 2012; PMID:22842478; <http://dx.doi.org/10.1038/nm.2883>
9. Näsman A, Andersson E, Nordfors C, Grün N, Johansson H, Munck-Wikland E, et al. MHC class I expression in HPV positive and negative tonsillar squamous cell carcinoma in correlation to clinical outcome. *Int J Cancer* 2013; 132:72-81; PMID:22592660; <http://dx.doi.org/10.1002/ijc.27635>
10. Leibowitz MS, Srivastava RM, Andrade Filho PA, Egloff AM, Wang L, Seethala RR, et al. SHP2 is overexpressed and inhibits pSTAT1-mediated APM component expression, T-cell attracting chemokine secretion, and CTL recognition in head and neck cancer cells. *Clin Cancer Res* 2013; 19:798-808; PMID:23363816; <http://dx.doi.org/10.1158/1078-0432.CCR-12-1517>