Establishment and characterization of a VX2 carcinoma derived rabbit cell line for the study of human papillomavirus associated head and neck cancer

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Introduction. For nearly 20 years, the auricular VX2 carcinoma of the New Zealand White (NZW) rabbit serves as an animal model for human and neck squamous cell carcinomas (HNSCC) (1). Our previous studies (2,3) demonstrated the suitability of the VX2 carcinoma as a model system for HPV associated HNSCC since this tumor, similarly to HPV+ HNSCC, is the consequence of a papillomavirus (cotton tail rabbit papilloma virus, CRPV) infection. Against this background it is of utmost importance to develop a VX2 carcinoma derived cell line to be able to perform in vitro studies with this animal model. Single reports (4,5,6) about already established VX2 cell lines refer either to discontinued or not readily available cell lines. The aim of this study was to develop a stable VX2 carcinoma derived cell line for studying tumor biological aspects of VX2 carcinoma cells in vitro that would not require the use of the VX2 carcinoma in vivo animal model.

Materials and Methods. VX2 tissue was obtained from VX2 tumor bearing NZW rabbits. The tumor tissue was minced and propagated in DMEM:F12 (1:1) media (7). Outgrowing cells were continuously sub cultured for more than 150 passages (Fig. 1). In situ hybridization of VX2 tumor appeared positive for viral transcripts (Fig. 2). Validation and characterization of the resulting VX2 carcinoma derived cell line was performed by flow cytometry (Fig. 3A and B, Fig. 5), Western blot analysis (Fig. 3C), fluorescence microscopy (Fig. 4) and qRT-PCR (Fig. 6).

Results. High passage numbers (>150) of VX2 carcinoma derived cells mainly excluded the presence of non-tumor cells and resulted in a highly proliferative cell line. Flow cytometry of these cells demonstrated the presence of two different subpopulations (Fig. 3). These populations were shown to represent two phenotypes of the same cell line since after single cell sorting of either of the population and further culturing both phenotypes reappeared (Fig. 3, S). Western blot analysis demonstrated expression of EGFR (epidermal growth factor receptor) in VX2 carcinoma derived cells demonstrating their epithelial origin. qRT-PCR demonstrated detectable transcript levels of CRPV E6 and E7 oncogenes (Fig. 6).

Conclusion. Having a matching VX2 tumor animal model and VX2 tumor cell line system for papillomavirus associated HNSCC will allow to evaluate diagnostic and therapeutic procedures in vitro that otherwise would have been done in vivo, thereby helping to reduce the required number of animal experiments.

References.

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