Establishment and characterization of a VX2 carcinoma derived rabbit cell line for the study of human papilloma virus 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 head 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 (cottontail 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 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 cells 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) 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 population as observed in Fig. 3B, this population was repeatedly subcultured according to Fig. 3C). In situ hybridization 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) qRT-PCR (Fig. 6).

Conclusion. Having a matching VX2 tumor animal model and VX2 tumor cell line system for papillomavirus associated HNSCC will allow to perform 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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