Gene Analysis of VX2 Carcinoma After Treatment with O₃/O₂-PP

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Introduction

Sequamous cell carcinomas of the head and neck region (HNSCC) frequently metastasize and show a high mortality rate in man and animals. Being an accepted animal model for studying the progression and metastatic spread of HNSCC, the highly aggressive and lethal VX2 avian carcinoma cell model of the New Zealand White (NZW) rabbit was applied in this study.

As is true for many cancers HNSCC tumor cells somehow evade the body's immune system. Therefore, being able to activate the immunostimulation toward HNSCC tumor cells should help in recognition and eradication of this tumor entity. Enhancing the immunoevasion capacity is an emerging concept in recent cancer immunotherapies particularly those focusing on immunomodulators or up-regulators of the immune response.

Ozone, recently found to be produced endogenously by granulocytes (Babior et al., PNAS, 2003), is a gas with complex influence on the radical biology in man and animals. Early in vitro studies described ozone as a radiomimetic gas, able to selectively inhibit growth of isolated human alveolar, uterine, breast, and endometrial carcinomas (Sweet et al., Science, 1980). Only studies concerning the anti-tumorigenic effect of an ozone/oxygen gas mixture in vivo revealed, that this gas mixture when intraperitoneally insufflated into VX2 carcinoma bearing rabbits resulted in a significant regression of the tumours and its associated metastases (Schulz et al., Int. J. Cancer, 2008).

In our current study we focused on the mechanisms underlying the observed anti-tumorigenic effect of O₃/O₂-PP. Methods. Four rabbits were inoculated with VX2 tumor cell suspension and tumour growth was allowed for 14 days as previously described. Then, two of the rabbits were treated with ozone/oxygen gas mixture (sham) with O₃/O₂-PP. Progressive tumors were harvested, RNA was extracted and mRNA was analyzed with the Trizol® method. Microarray analysis was performed with the Affymetrix GeneChip Expression 44K Array (ID: 00208) from Affymetrix at the EMBL GeneCore Facility (European Molecular Biology Laboratory, Heidelberg, Germany).

The aim of the present study was to further analyse the mechanisms underlying the observed anti-tumorigenic effect of O₃/O₂-PP. Objectives. The aim of the present study was to further analyse the mechanisms underlying the observed anti-tumorigenic effect of O₃/O₂-PP. RESULTS. In the state of O₃/O₂-PP-induced remission to unexplained possible underlying mechanisms of the anti-tumorigenic effect.

Methods and Materials

Experimental groups: i) animals which received an O₃/O₂-gas mixture therapy ii) animals which were sham-treated (no gas insufflation).

Tumor induction: A VX2 tumor cell suspension was inoculated in the right ear and tumor growth was allowed for fourteen days.

Therapeutical treatment: On day 14, the daily therapeutic treatments with O₃/O₂-gas mixture (5%/95%) were initiated lasting for a period of five days. Animals of the sham group were connected to the Medizub Orca processor, but received no gas insufflation.

Gene array: The micro array chip used in this study is a new commercial chip designed by Affymetrix. It is a 44K Chip representing 42,034 genes (product ID: 00208), and based on gene informations obtained from the database RefSeq (Release 20 May 2008), according to IEU (Build 11, March 2008) and Entrez (Release 40, Feb 2005), isolated total RNA was verified regarding its quality and subsequently processed by the microarray unit at the European Molecular Biology Laboratory (EMBL, Heidelberg).

The study was in accordance with the guidelines of FELASA, approved by the NZW Gas (V 54-19 20-15/1) MR, Nr. 24.2005.

Reference


Result

Figure 1. Effect of O₃/O₂-PP treatment on VX2 tumor progression and gene expression. A: Typical course of VX2 tumor remission after O₃/O₂-PP treatment. B: Shown are the two tumors in remission 47 days after initial treatment and the two untreated tumors under progression (A, B) at the stages at which these tumors were used for the gene array C.

Figure 2. Gene profile analysis reveals several immune relevant candidate genes. Several differently regulated genes could be identified. Particularly immune-related genes appear up-regulated. Abbreviations and functions: CD4 and CD8 T cell receptor co-receptors for T helper activity (CD4) and T cytotoxic activity (CD8); CD3: T cell receptor co-receptors for thymocyte selection and activation; CD14: endotoxin receptor on monocytes/macrophages in association with the TLR4: TLR4: endotoxin receptor expressed by monocytes/macrophages CCR10: chemokine (C-C motif) receptor 10; CR1: Complement receptor 1; TLR: Toll-like receptor; EGFR: epidermal growth factor receptor; eNOS: endothelial nitric oxide synthase; SELL: sialyl Lewis X molecule: 1; VCAM-1: Vascular cell adhesion molecule-1.

Conclusion

O₃/O₂-PP treatment: • Effectively eradicates a highly metatizing cancer at a high rate. • Appears to mediate its anti-tumorigenic effect in the body's own immune system.

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References

