Influence of local environment and differentiation on experimental prostate cancers on tumor metabolism and vascularisation

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Introduction

Mortality of patients with prostate cancer is only 5%. Unfortunately the selection of patients who will develop a highly infiltrating and metastasising tumor is currently not possible. Thus, the aim of this study was to investigate the influence of local environment on the development of experimental prostate cancer of different malignancy in rats in order to simulate primary tumor and metastasis. All tumors were evaluated with histological and immunhistological methods. For this purpose in agreement with clinical examination protocols, morphologic MRI, DCE-MRI and ^{1}H-MRS were applied. Non-invasive imaging was supplemented by histological evaluation and immunofluorescence analysis.

Material and methods

Tumors were induced by implanting H- (hormone-sensitive), HI- (hormone-insensitive) and AT1- (anaplastic) Dunning prostate cancer fragments [1] subcutaneously or orthotopically in the prostate of Copenhagen rats [2]. Taking 3 diameters from morphologic T_2-weighted MR-images tumor growth was monitored over a time period of up to 264 days, except for rats that were sacrificed due to excessive tumor growth. MRI was performed at a clinical 1.5-Tesla MR system using an animal resonator. A T_1-weighted SRTF sequence (temporal resolution: 0.75s) was used to obtain signal-time courses upon administration of Gd-DTPA (B_0 = 1.5T). Postprocessing was done using a two-compartment model considering the individual arterial input function [3]. Single-voxel ^{1}H MR spectroscopy (PRESS, voxel size 4×4×4 mm^3, T_E = 14 ms, T_R = 2000 ms, measurement time 4:24 min) was performed for subcutaneous tumors at 9.4T (BioSpec® 94/20, Bruker, Ettlingen, Germany). Histological evaluation of tumors included HE and immunofluorescence staining (CD31, Ki67, Tunel and smooth muscle actin).

Results

Tumor growth depended on the tumor-subtype and the implantation site. Particularly the low malignant H-tumor grew significantly slower orthotopically (p<0.001) and had reached mean tumor volumes of less than 26 ml after 264 days while subcutaneous tumors had to be removed within 174 days due to a size of more than 172 ml (Fig.1 left). The more malignant tumor subtypes (HI and AT1) grew significantly slower and showed less pronounced differences in growth depending on the implantation site. Surprisingly, metastases were only observed in lymph nodes of the intermediate malignant orthotopic HI-tumors. DCE-MRI showed high intra-individual variance. Blood flow was significantly reduced in orthotopic H-tumors as compared to subcutaneous ones (p<0.05) as well as the other subtypes with different malignancy. Relative blood volume and surface-area permeability product did not significantly differ between orthotopic and subcutaneous tumors and the different malignant subtypes, respectively. All tumor subtypes showed elevated signal intensity ratios of choline-creatine in ^{1}H MRS (Fig.1 right, range in the displayed spectra: I_{Cho}/I_{Cr} ~ 1.1-1.6). A more anaplastic tumor phenotype was related to enhanced signals of free fatty acids. While orthotopic H-tumors appeared highly differentiated with tubular structures, the subcutaneous ones showed multifocal dedifferentiated tumor areas with high tumor cell polymorphy and dense tumor cell clustering. Immunofluorescence evaluation revealed elevated vessel density (CD31) and a significantly higher degree of smooth muscle actin positive mature vessels in orthotopic H-tumors as compared with subcutaneous H- and all other tumors. As a trend, a higher degree of apoptosis was found for all orthotopic tumors as compared with the subcutaneous ones, which was significant for H-tumors.

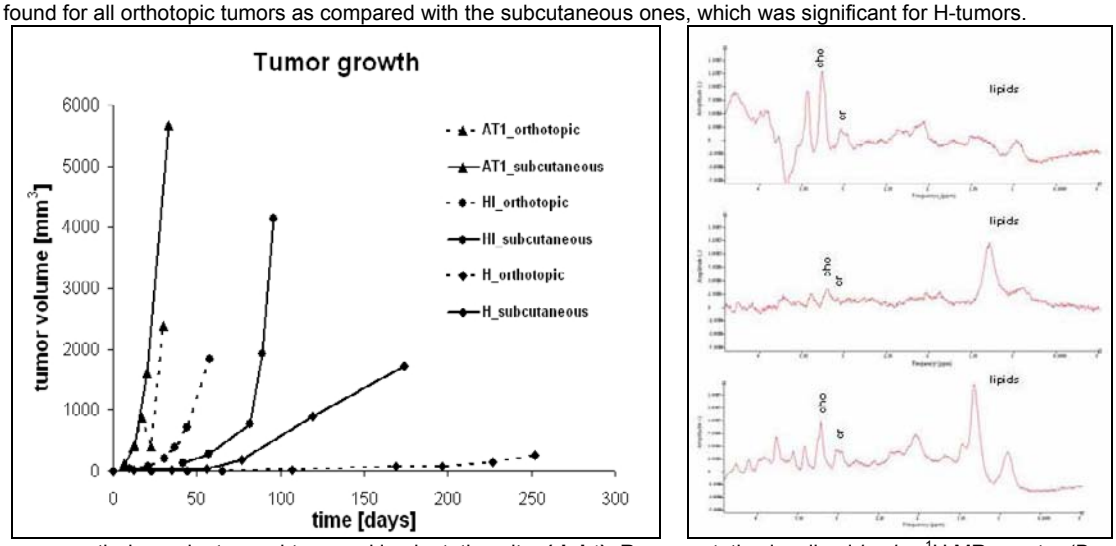

Fig. 1 (left): Tumor growth dependant on subtype and implantation site. **(right):** Representative localized *in vivo* ^{1}H MR spectra (B_0 = 9.4 T) of H–, HI– and AT1–tumors show increasing lipid components in more dedifferentiated tumors.

Discussion

Orthotopic implantation reduces growth of low malignant prostate cancers. This may be attributed to an interaction of the tumor with the surrounding tissue preserving the high differentiation of tumor cells and keeping a balance of proliferation and apoptosis. In contrast, highly malignant tumors are more dedifferentiated from the very first and do not show these pronounced differences. Most probably due to the slow growth orthotopic H-tumors develop a more differentiated vascularisation, which was reflected in a higher degree of mature vessels. In this context, a lower amount of shunt perfusions may explain the reduced perfusion in orthotopic H-tumors. Choline-creatine intensity ratios were enhanced in all tumors and the higher level of free fatty acids in more dedifferentiated tumors can be explained by increased membrane turnover and cell regression leading to micro-necroses.

References: [1] Isaac JT et al. Prostate 1986 9:261-87; [2] Kiessling et al. Invest Radiol 2004 39(1):34-44 [3] Brix et al. MRM 2004 52(2)420-9
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