THE EFFECT OF TUMOR PROTEASES ON INVASION AND VASCULARISATION

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Introduction:

Matrix-Metallo-Proteinases (MMPs) are a group of enzymes that are responsible for the degradation of extracellular matrix proteins during angiogenesis and thus essential for tumor growth and spread. The focus of this study was to analyze the effects of two different MMP inhibitors (*Ro28-2653* and *Ag3340)* on vascularisation and invasion of skin carcinomas in vitro and in vivo.

Methods:

In vitro: High-grade malignant human squamous carcinoma cells (HaCaT-ras A5RT3) were cultivated in a skin organotypic 3D-coculture (3D-OTC) model at the air-liquid interface on a dermal equivalent consisting of collagen type I with embedded normal human dermal fibroblasts. Cultures were treated with an MMP inhibitor (MMPI) *Ro28-2653* (20mM) or with solvent alone. OTC samples were analyzed by frozen sections and additionally conditioned media were collected for protein-analysis by ELISA.

In vivo: 6 HaCaT-ras A5RT3 tumor xenografts in nude mice were treated with the MMPI *Ag3340* for 6 days (5 ml/kg/day). 5 mice remained untreated (controls). All MRI experiments were carried out on a clinical 1.5 T whole-body MRI system (Siemens Magnetom Vision, Erlangen, Germany) using a custom-made small animal Tx/Rx radiofrequency coil. Vessel Size Imaging was applied, which bases on the measurement of susceptibility differences between vessels and the surrounding tissue induced by injection of an intravascular superparamagnetic contrast agent (CA). The vessel size index R can then be calculated according to the formula proposed by Troprès and coworkers [1]:

$$R = 0.425 \cdot \left(\frac{D}{\gamma \Delta \chi B_0} \right)^{1/2} \left(\frac{\Delta R_2^*}{\Delta R_2} \right)^{3/2}$$

This formula assumes a diffusion coefficient D of 10-3 mm²/s and a susceptibility difference $\Delta\chi$ of 0.571 ppm [1]. $\Delta R2$ was estimated from the signal ratio of T2w images (SE, TR 6000ms, TE 100ms, average 1, FOV 62x50, matrix 128x104) and $\Delta R2^*$ was calculated by quantifying T2* (FLASH 2D, TR 320, TE 4,76-47,6ms (10 Echos before and 8 after CA administration), flip angle = 45°, averages 3, FOV 62x50, matrix 104x128). Both sequences were performed before and after CA administration (Very Small Superparamagnetic Iron Oxide Nanoparticles, VSOP, 12µl/mouse, Ferropharm, Teltow). In addition high frequency volumetric Power Doppler ultrasound (HF-VPDU) examinations were carried out using a microultrasound system (Vevo 770, Visualsonics Inc., Toronto, Kanada) operating at a Doppler frequency of 30 MHz. The micro vessel density was observed by measuring the colour pixel density (CPD). Differences in vessel density and maturity between treated and untreated tumors were also confirmed by immunofluorescence (IF) microscopy measuring area fractions of vessels (CD31) and pericytes (Ng2).

Results:

When analyzing the effect of MMPI on tumor invasion in the 3D-OTC model, we observed a clear reduction of invasion in the treated samples. While in untreated OTCs 70% of the collagengel was degraded within 21 days (d) treated samples showed only a degradation of 40% during the same time window (*Fig.1*). This reduction in collagenolytic activity coincided with a striking difference in the amount of the angiogenic growth factor VEGF and the vessel maturation factor PDGF. At d14 und d21 upon treatment the expression of PDGF was up regulated reaching values of 50% above the untreated samples. In contrast VEGF was down regulated during the first two weeks of *Ro28-2653* treatment and reached the level of controls at d21 (*Fig.2*). This alteration in the expression of angiogenic and vessel maturation factors that was observed upon MMPI (*Ro28-2653*) in vitro could be one reason for the effect of MMPI (*Ag3340*) in tumor vascularisation that we observed in vivo. Six days of treatment with *Ag3340* did not yet resulted in significant changes on tumor volume (untreated: 3.00 ± 3.2mm^3, treated: 3.5 ± 1.33mm^3). At the same time vascularity, analyzed by ultrasound, tended to be lower upon treatment (0.84 ± 0.76CPD) as compared to controls (1.33 ± 0.6CPD). The mean vessel diameter, analyzed by VSI, increased upon 6d of *Ag3340* treatment (pre CA: 40 ± 10µm, post CA: 70 ± 10µm) while staying constant in untreated animals (pre CA: 53 ± 10µm, post CA: 57 ± 20µm) (*Fig.3*). Immunfluorescence analysis (IF) showed, that the amount of apoptotic tumor cells and CD31-positive vascular cells decreased upon *Ag3340* treatment while the expression of Ng2, a marker for vessel pericytes, tended to be weaker (*Fig.4*).

Discussion:

Treatment with MMP inhibitors reduces gelatinolytic activity in OTC in vitro and vascularity in vivo. Interestingly the treatment with *Ro28-2653* up regulates the protein expression of the vessel maturation factor PDGF, while the angiogenic factor VEGF is down regulated. This suggests a growth factor profile that mediates stromal maturation with a down regulation of angiogenesis. In agreement with this hypothesis, we observed in vivo an increase of the mean vessel size diameter, while total vascularity decreased. Upon MMP treatment with *Ag3340* further histological analysis confirmed, that the increasing mean tumor vessel diameter is caused by vessel regression and maturation and might be triggered by the change in VEGF und PDGF expression that we observed in vitro upon *Ro28-2653* treatment.

Acknowledgement:

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Reference:

[1] Troprès I. et. al., Vessel Size Imaging, Magn Reson Med 2001; 45:397-408.

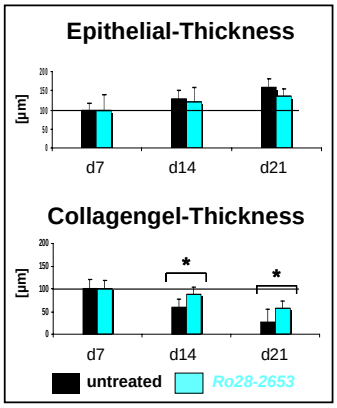

Fig.1: *Epithel- and Collagengel-Thickness of OTCs in % upon MMPI treatment. *: P<0.05.*

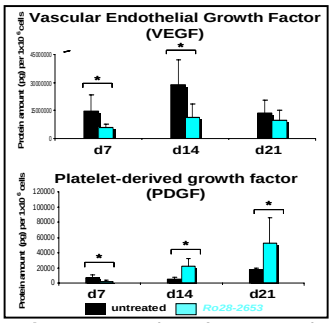

Fig.2*: Expression of VEGF and PDGF in untreated and treated in vitro. *: P<0.05.*

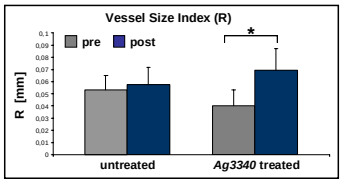

Fig.3*: Changes of R in untreated and treated tumors, *: P<0.05.*

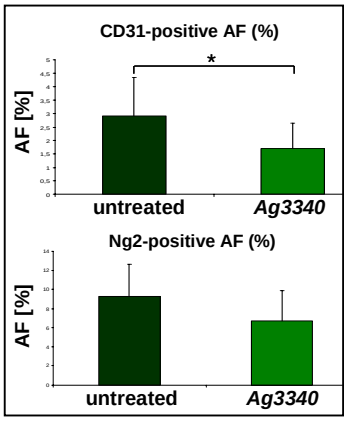

Fig.4*: Changes of CD31and Ng2 positive areafractions (AF) under treatment shown by IF staining analyses.*: P<0.05.*