High-Dose Ascorbate induces the ROS-driven mitochondrial Cell Death Pathway in Melanoma Cells

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(A) Western blot analyses of BLM cells 4 h and 8 h after treatment with 200 µM or 8 mM ascorbate. Treatment with 200 µM ascorbate showed no effect, whereas 8 mM ascorbate completely down-regulated the expression of HIF1α, GLUT-1 and oncogenic pathways active in melanoma (NFκB, pS6P (mTOR), Akt) within 4 h. (B) Total ascorbic acid was determined in native tissues via HPLC, and increased concentrations were found in s.c. metastases compared to skin, nevi and other skin tumors (7.243 µM±1.056 µM vs. 0.741 µM±0.222 µM, p=0.0244; vs. 2.64 µM±0.141 µM, p=0.041; vs. 2.062 µM±0.468 µM, p=0.011; t-test). (C) Tissue microarray stained for the copper-binding protein metallothionein, GLUT-1 and HIF-1α. The expression of all three proteins increased from nevus to primary melanomas and metastases. (D, E) Kaplan-Meier plots of patients with primary melanomas with long-term follow-up data (median 97.5, 1-186 months) demonstrate a reduced cumulative survival for metallothionein (n=250) (negative vs. positive expression, p=0.017) and for GLUT-1 (n=258) (low vs. high expression, p=0.002; log-rank test). (F) µX-ray-fluorescence spectroscopy (rectangular insert) was applied to measure the copper concentration in H&E-stained sections of 70 primary melanomas and metastases of the TMA. The copper concentration significantly increased from thin primary melanomas (<1 mm) to thick melanomas (>4 mm) to metastases (73% increase, p<0.001, t-test).

Figure 1: The regulation of HIF1α, GLUT-1, NFκB, the mTOR pathway and Akt in melanoma cells by ascorbate.
Methods

**Analysis of copper by synchrotron radiation µ-X-ray fluorescence (SR-µXRF)**
The analysis of copper distribution in primary melanomas and metastases was performed on a 10-µM section of the melanoma tissue microarray described below. SR-µXRF measurements were performed at beamline L (HASYLAB, Hamburg, Germany) using a multilayer monochromator crystal pair as described previously. Briefly, the energy of the incoming X-ray beam was set to 16.6 keV, and a polycapillary optic was used to focus the X-ray beam to approximately 15 µm in diameter. Fluorescence analysis was performed at 90° to the incoming X-ray beam in the plane of polarization to optimize the signal-to-background ratio. A Vortex SDD detector (SII NanoTechnology USA Inc.) equipped with a Mo-collimator was applied to measure the X-ray fluorescence. Two-dimensional fluorescence maps of the samples were collected with a step size of 10 µm in both directions and an acquisition time of 10 s per point. An analysis of the raw data was performed using AXIL.

References