Quantitative imaging of hypoxic CAIX+ areas in syngeneic mouse models

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Abstract

Intro Limited diffusion of oxygen in combination with increased oxygen consumption leads to chronic hypoxia in most solid cancers. This scarcity of oxygen is known to induce radioresistance, but also leads to a more immunosuppressive immune phenotype. CAIX is an enzyme catalyzing acid export in hypoxic cells upregulated by HIF-1α, and is an endogenous biomarker for hypoxia. We developed a radiotracer that recognizes murine CAIX ([111In]In-DTPA-mCAIX) to visualize chronic hypoxia in syngeneic mouse models.

M&M Three syngeneic tumor models were used in this study (B16ova, B16F1, MOC1). Cells were cultured at 1% O2 and CAIX expression was determined by FACS analysis or cells were incubated with 700 Bq 111In-anti-mCAIX and cell-associated activity was measured by radioactivity counting. *In vivo* distribution of tracer uptake and CAIX expression were visualized using autoradiography and immunohistochemistry. Image analysis was performed by parametric mapping in ImageJ. In vivo SPECT/CT scans were acquired using the mouse HS 1.0 mm pinhole collimator (U-SPECTII/CT) or the GP-M 0.60 mm pinhole collimator (U-SPECT6/CT), followed by a CT scan. Scans were analyzed using VivoQuant.

Results In vitro, inducibility of CAIX expression was determined for several syngeneic tumor models (B16ova;high, B16F1;intermediate, MOC1;low). [In111]-DTPA-mCAIX showed specific binding to B16ova cells when cultured at 1% O2 (5.7±0.9%), but not at 20% O2 (0.02±0.3%). In vivo, CAIX could be visualized by SPECT using [In111]-DTPA-mCAIX(Fig 1A). Autoradiography and immunohistochemistry of tumor sections showed a strong spatial correlation of CAIX with [In111]-DTPA-mCAIX for CAIX+ tumor models (B16ova; r=0.55±0.21, B16F1; r=0.46±0.12) but not for CAIX- tumors (MOC1; r=-0.20±0.17)(Fig1B). Quantification of CAIX+ fraction on SPECT/CT images showed significant higher fraction in B16ova (0.18±0.03) compared to B16F1 (0.07±0.05) and MOC1 (0.03±0.04) tumors(Fig1C).

Conclusion The hypoxia related marker CAIX can be used to visualize hypoxic areas in syngeneic mouse models using the SPECT-radiotracer [In111]-DTPA-mCAIX. We show this technique is able to distinguish CAIX high from CAIX low tumors by ex vivo analysis and in vivo SPECT/CT imaging. In the future, this technique could be used to distinguish hypoxic from non-hypoxic tumors before or during hypoxia targeted or reducing treatment and thereby help optimizing this strategy to improve immuno- and radiotherapy efficacy in preclinical models.

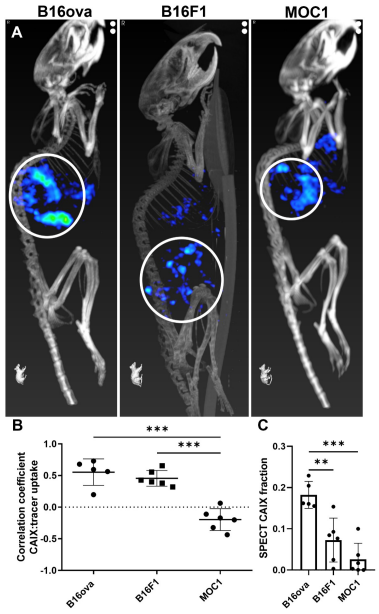

Figure 1. 111In–anti–mCAIX specifically accumulates in CAIX+ tumors and can be used to discriminate between low from high expressing tumor models by microSPECT/CT. (A) Maximum intensity projections of 111In–anti–mCAIX microSPECT/CT images of several tumor models. Tumor indicated by white circle (B) Spatial correlation of CAIX expression with intratumoral tracer uptake as determined by co-registration analysis of autoradiography and immunohistochemistry. (C) CAIX+ fraction of tumors determined by SPECT quantification.