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Title: Hypoxic phenotype in pulmonary metastases of different primary tumors

Mr. Thomas 25702 Schweiger thomas.schweiger@meduniwien.ac.at [1,2], Mr. Christoph 25703 Nikolowsky christoph.nikolowsky@meduniwien.ac.at [1,2], Mr. Lukas 25704 Lehmann lukas.lehmann@meduniwien.ac.at [1], Mr. Robert 25705 Wiebringhaus robert.wiebringhaus@meduniwien.ac.at [1], Prof. Dr György 25706 Lang gyoergy.lang@meduniwien.ac.at [1], Prof. Dr Peter 25707 Birner peter.birner@meduniwien.ac.at [3], Prof. Dr Walter 25708 Klepetko walter.klepetko@meduniwien.ac.at [1], Prof. Dr Hendrik-Jan 25709 Ankersmit hendrik.ankersmit@meduniwien.ac.at [1,2] and Prof. Dr Konrad 25710 Hoetzenecker konrad.hoetzenecker@meduniwien.ac.at [1,2]. [1] Department of Surgery - Division of Thoracic Surgery, Medical University of Vienna, Austria, 1090 ; [2] Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria, 1090 and [3] Clinical Institute of Pathology, Medical University of Vienna, Austria, 1090 .

Body: Tumor hypoxia has been shown to be a common feature in tumor growth and metastasis. It negatively affects the clinical outcome in patients with various malignancies. Although hypoxia is described in many primary tumor types, data on pulmonary metastases is lacking. We determined the expression of hypoxia-related proteins in paraffin-embedded specimens of pulmonary metastases of different types of cancer (breast cancer n= 6, colo-rectal carcinoma n=29, renal cell carcinoma n=13, sarcoma n=10). All recruited patients underwent curative metastasectomy at the Department of Thoracic Surgery, MUV, between April 2009 and December 2011. Expression of carbonic anhydrase 9 (CA9), heat-shock protein 70 and HIF prolyl hydroxylase 2 was evaluated by immunohistochemistry. Metastasis free survival and estimated tumor size and overall survival was determined for all sub-groups. Hypoxia related proteins are expressed in 66.6%, 76.9%, 7.7% and 0.0% of pulmonary metastases of breast cancer, colo-rectal carcinoma, renal cell carcinoma and sarcoma, respectively. Furthermore, metastases with highly positive CA9-staining are associated with early tumor spreading to the lung (Disease free interval: 21.8±7.0 months vs. 46.1±26.6 months; p=0.008) This study provides first evidence for hypoxia marker expression in pulmonary metastases and its clinical relevance. These findings may be important for future therapeutic targets in the therapy of generalized malignant diseases.