# VASCULAR DENSITY CORRELATES WITH HbNO SIGNALS IN MURINE TUMORS

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**Electron paramagnetic resonance (EPR) enables detection of nitric oxide in tumors, due to stable paramagnetic complexes of NO with heme and non-heme Fe(II). Hemoglobin is the main source of heme iron and occurs in the tumor tissue together with growing blood vessels. We analyzed formation of two kinds of nitric oxide complexes: HbNO and $Fe(DETC)_2NO$ in solid tumors with different vascularization. L5178Y lymphoma and Ehrlich carcinoma (EC) solid tumors were inoculated s.c. in Swiss mice. Animals were injected i.p. with the exogenous spin trap, sodium diethyldithiocarbamate (DETC), and sacrificed after 30 min. No exogenous iron (II) was applied. EPR signals of HbNO and $Fe(DETC)_2NO$ complexes were measured in excised frozen tumor tissue by X-band spectrometer at 77K. Tumor vessels were stained immunohistochemically using CD31 as an endothelium marker of vascular density (MVD) was quantified as number of vessels per $mm^2$.**

**NO generation increased with time and reached similar level in both tumor types, as shown by $Fe(DETC)_2NO$ complexes. In L5178Y HbNO level increased in time together with gradual increase of $Fe(DETC)_2NO$ complexes, whereas in EC the level of HbNO was constantly low (about 5-fold lower than those of lymphoma) and was not correlated with the amount of $Fe(DETC)_2NO$. This result was consistent with vascular density estimation. MVD was 10-fold lower in EC tumors than in L5178Y tumors.**

**Conclusions: EPR signal of endogenous HbNO complexes reflects nitric oxide production only in well-vascularized tumors. If signals of HbNO complexes are relatively low and not correlated with strong $Fe(DETC)_2NO$ signals, HbNO reflects the quantity of available hemoglobin in the tumor tissue and indirectly estimates vascularization. Spin trapping of NO using DETC is a better measure of NO production in the tumor tissue.**