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LS-11-P-5899 Association of protein kinase B (Akt) isoforms with subcellular compartments in the left and right ventricles of rat heart adapted to hypoxia

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Chronic hypoxia results in pulmonary hypertension and right ventricle hypertrophy. On the other hand, it increases myocardial resistance to acute ischemia/reperfusion injury (Kolar et al., 2007). Akt signalling plays an important role in cardioprotective mechanisms (Ravingerova et al., 2007). Akt1 and Akt2 are known as major isoforms and their localization in cardiomyocytes appear to be crucial for their action in the area of mitochondria and sarcolemma. We aimed to assess the subcellular localization of Akt1 and Akt2 and their co-localization with either mitochondria or sarcolemma by quantitative fluorescence microscopy in hearts of rats adapted for 3 weeks to intermittent hypobaric hypoxia (7000 m, 8h/day). Frozen section method of indirect immuno-fluorescence staining combined with marker counterstaining was used. Cryosections were incubated with rabbit primary polyclonal antibodies against rat Akt1 (Cell Signalling) and Akt2 (GenScript). As counterstaining, either mitochondrial compartment or sarcolemma markers were used. Mitochondria were stained with MitoProfile Total OXPHOS Rodent Antibody Cocktail (Abcam), WGA-TMRM conjugate (Molecular Probes) was used as sarcolemma marker. Pearson correlation coefficients indicating the level of colocalization of both Akt isoforms with the two subcellular compartments were calculated using ICA plugin of Fiji ImageJ software.

Our results showed thatAkt2 was not associated with sarcolemma and its association with mitochondrial compartment was higher compared to Akt1 in both ventricles. Adaptation to hypoxia decreased ($p<0.005$, $n=6$) mitochondrial association of Akt1 in the hypertrophied right ventricle only while increasing its association with sarcolemma in both ventricles.

We conclude that Akt1, but not Akt2, subcellular localization is affected by chronic hypoxia with significant differences between right and left ventricles.

Ostadal B, Kolar F. Cardiac adaptation to chronic high-altitude hypoxia: beneficial and adverse effects. Respir Physiol Neurobiol. 2007 Sep 30;158(2-3):224-36.

Ravingerova T, Matejikova J, Neckar J, Andelova E, Kolar F. Differential role of PI3K/Akt pathway in the infarct size limitation and antiarrhythmic protection in the rat heart. Mol Cell Biochem. 2007 Mar;297(1-2):111-20.

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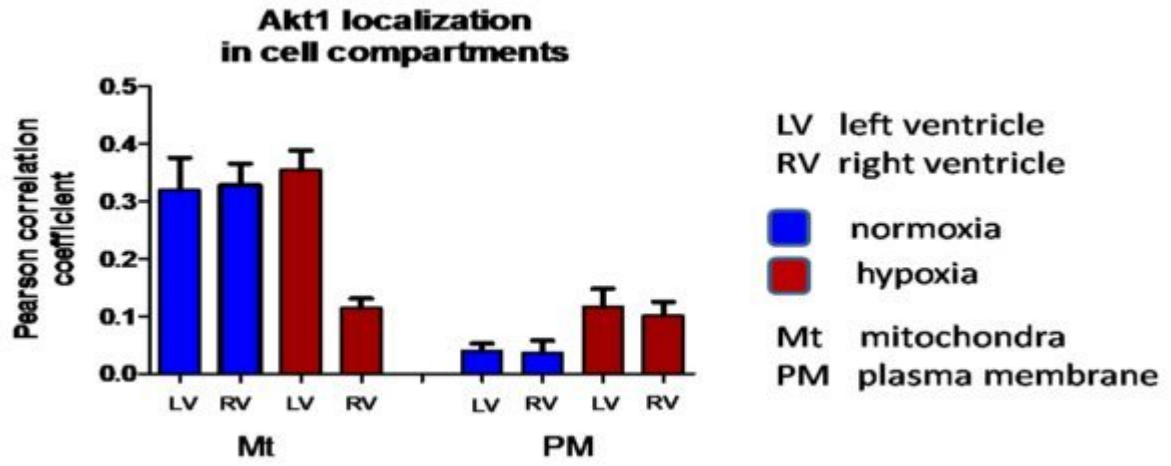

Fig. 1: The overall pattern of Akt1 fluorescent signal in different cell compartments. Akt1 isoform is more associated with mitochondria than with sarcolemma. Under hypoxic condition Akt1 detach from mitochondria in RV ($p<0.0022$, $n=6$) and translocate to plasma membrane in both ventricles ($p < 0.0001$, $n=6$).

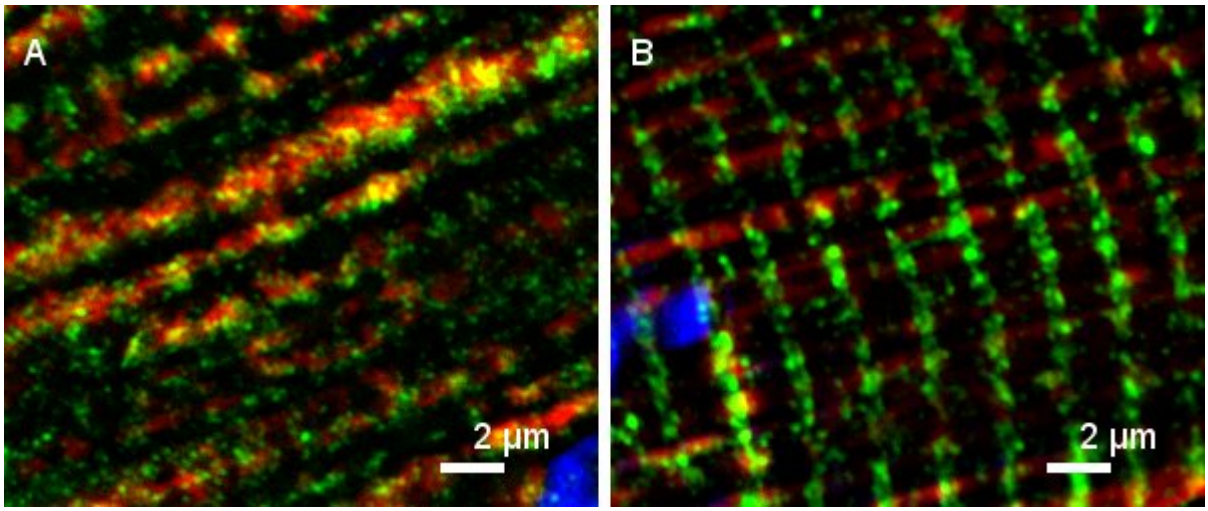

Fig. 2: Akt1 colocalization with mitochondria in hypertrophied right ventricle of rat. After adaptation to hypoxia Akt1 isoform (green) detached from mitochondria (red) in right ventricle ($p<0.0022$, $n=6$). A: tissue section of control samples B: sections of samples adapted to hypoxia

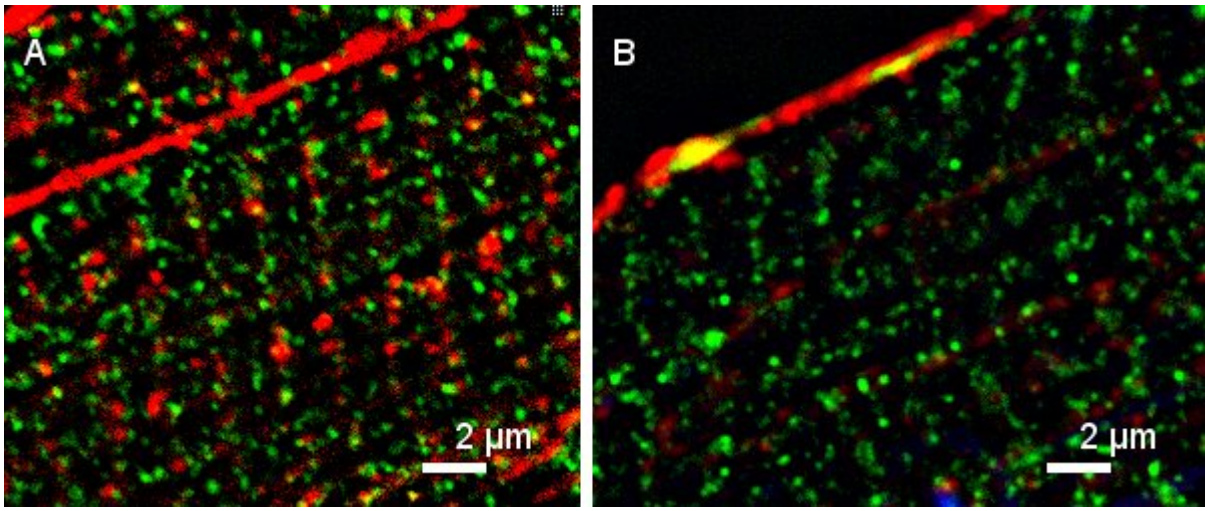

Fig. 3: Akt1 colocalization with plasma membrane. After adaptation to hypoxia, Akt1 (green) seems to translocate to plasma membrane (red). Pearson's coefficient increased significantly ($p < 0.0001$, $n=6$). Nevertheless the absolute values of coefficient are very low and further evidence is necessary. A: control sample B: sample adapted to hypoxia