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ATTENUATION OF AIRWAY RESPONSIVENESS IN PAK 1 KO MICE.

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Pak 1 (p21-activated kinase) is involved in the regulation of airway smooth muscle (ASM) contraction *in vitro*. We hypothesized that the genetic disruption of *Pak1* would attenuate *in vivo* airway responsiveness to acetylcholine (ACh) in non-sensitized mice. Male C57B/6 wild type (WT) and *Pak1* knockout (KO) mice were anesthetized, tracheostomized, and ventilated with a computer-controlled small-animal mechanical ventilator (flexiVent, SCIREQ, Montreal, Quebec) with tidal volume of 10 mL/kg, PEEP of 4 cmH_2O, and rate of 150-170 breaths per minute. Resistance was measured with a 2.5 Hz, 1-second sine wave oscillation. Following determination of baseline resistance, measurements were repeated every 30 seconds for 5 minutes following each aerosol of ACh (0.3, 1, 3, 5, 7, 10, 33, and 50 mg/mL) delivered via an in-line nebulizer. Baseline airway resistance was similar for WT and *Pak1* KO mice (0.595 vs. 0.594 $cmH_2O.s/mL$; p= 0.75). Median airway resistance achieved statistical significance at 7 mg/mL (1.52 vs. 0.85 $cmH_2O.s/mL$; p = 0.032). The mean maximal resistance following ACh challenge was greater for WT compared to *Pak1* KO mice (3.4 vs. 1.6 $cmH_2O.s/mL$; p = 0.0005). Isolated tracheas from WT and *Pak1* KO mice were then excised, mounted securely to steel cannulas on each end and placed in a tissue bath containing physiologic saline solution (PSS) at 37C (flexiVent IAM, SCIREQ, Montreal, Quebec). The lumen of the airway and tubing connected to a pressure transducer were filled with PSS. A computer controlled syringe was used to raise the initial transmural pressure to 4.5 cm H_20. Pressure was recorded during isovolumetric contractions via increasing doses of acetylcholine (10^{-8} M – 10^{-4} M). Mean pressure generation by the isolated tracheas was greater for WT compared to *Pak1* KO at the first and second doses (10^{-8} M: 8.45 vs. 4.425 cmH_2O, p= 0.0136; 10^{-7} M: 8.73 vs. 4.9 cmH_2O, p= 0.031). There was no difference in maximal pressure generation for WT and *Pak1* KO tracheas (10^{-4} M: 11.01 vs. 11.12 cmH_2O; p= 0.92), indicating a decreased sensitivity to acetylcholine mediated constriction of airway smooth muscle in *Pak1* KO mice. We conclude that Pak1 is an important determinant of airway responsiveness *in vivo* and *in vitro*. Furthermore, these findings are a direct effect of decreased sensitivity by *Pak1* KO mouse airway smooth muscle to acetylcholine.

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NEUROFIBROMIN-DEFICIENT SCHWANN CELLS HAVE INCREASED LYSOPHOSPHATIDIC ACID DEPENDENT SURVIVAL AND MIGRATION – IMPLICATIONS FOR INCREASED NEUROFIBROMA FORMATION AND GROWTH DURING PREGNANCY.

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Background: During pregnancy, neurofibromas often enlarge or develop for the first time in females with neurofibromatosis type 1 (NF1). Lysophosphatidic acid (LPA) is a prototypic lysophospholipid that has been implicated in tumor progression. LPA modulates cell migration and survival of Schwann cells (SCs), and interestingly, LPA is made in increasing concentrations throughout pregnancy. SCs are the tumorigenic cells in the development of neurofibromas in NF1. Given the temporal nature of LPA production and neurofibroma formation during pregnancy, we hypothesized that LPA may be a candidate molecule that promotes Schwann cell (SC) migration and survival and potentially plays a role in the increase in neurofibroma formation during pregnancy. **Purpose:** To define the potential role of LPA on the biochemical and cellular functions of *Nf1-/-* SCs. **Methods:** Murine SCs were isolated from WT and *Nf1-/-* dorsal root ganglia at embryonic day 13.5 and cultured in media containing glial growth factor. To measure SC motility, confluent SC monolayers were wounded and wound closure was monitored by time lapse microscopy following LPA stimulation. Flow cytometry (FACS) was used to quantitate the relative amount of filamentous actin per SC after LPA stimulation. LPA-dependent survival was measured by FACS using Annexin V/PI staining, and PI-3K activity was assessed by measuring Akt phosphorylation. Ras and small RhoGTPase effectors were evaluated by Western blot. **Results:** LPA preferentially promoted Ras-mediated *Nf1-/-* SC survival, migration, and F-actin reorganization and polymerization in *Nf1-/-* SCs as compared with WT SCs. LPA induced hyperactivation of Ras and its downstream effectors, Akt and Rac1. Addition of LY0294002, a potent PI-3K inhibitor, significantly reduced SC survival and migration in both WT and *Nf1-/-* cultures. **Conclusions:** We demonstrate that there is a gain in functions of LPA-mediated migration and survival of *Nf1-/-* SCs, which is dependent on the Ras/PI-3K signaling axis. As Ras is an intractable molecular target, inhibition of the PI-3K pathway may be a potential therapy for neurofibroma development and progression in NF1 patients. Furthermore, LPA is a candidate phospholipid that modulates excessive cell survival and invasiveness of *Nf1-/-* Schwann cells that potentially may contribute to neurofibroma development and progression in pregnant women with NF1.