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Pre-clinical identification of potential molecular diagnostic biomarkers of secondary ischemia in microvascular fasciocutaneous flaps

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Compromise of surgical microvascular flaps due to acute or late post-operative circulatory complications remains a significant clinical problem worldwide. Timely detection of vessel occlusion may salvage a flap from necrosis, but monitoring of physical flap appearance is not always feasible and the etiology of compromise is hard to identify. Here we used a translational laboratory animal model of abdominal wall repair and Affymetrix gene expression microarray platform to reveal the molecular tissue and wound fluid markers of the arterial (AO), venous (VO) or mixed arterio-venous (AVO) thrombosis of the pedicled flap *in vivo*. Bilateral 7x2.5cm epigastric flaps were raised in ~12 month old male and female Sprague-Dawley retired breeder rats. Baseline fasciocutaneous tissue sample biopsies were obtained from the upper abdominal quadrants of sixteen sex-matched rats prior to flap elevation. All flaps were then subjected to primary ischemia (PI), which was induced for 4 h by temporary clamping of superficial inferior epigastric artery (SIEA) and vein (SIEV) vessels to mimic free tissue transfer and anastomosis. Normoxic control (NC) samples were obtained from flaps after 24 h of non-obstructed reperfusion. AO, VO or AVO samples were retrieved from paired flaps undergoing secondary ischemia (SI), in which SIEA, SIEV or both vessels were ligated for 4 h following 20 h of reperfusion. Total RNA was isolated from individual animal samples (n=8/gender group) and then pooled at equal concentrations prior to assessment of RNA quality. We observed 5011 (in males) and 4457 (in females) differentially expressed genes (DEGs) that demonstrated more than 2-fold change in NC versus baseline conditions. There were 1403, 1643 and 2351 DEGs in male AO, VO and AVO flaps when compared to NC. In females, AO, VO and AVO-associated DEG numbers were smaller (1074, 828 and 1518, respectively). Independently of gender, downregulation of keratinocyte-specific small proline-rich protein 4 (Sprr4) and upregulation of ankyrin repeat domain 37 (Ankrd37), previously identified as a target of hypoxia inducible factor-1 (HIF-1) gene, was significantly associated with the arterial SI. Venous SI was characterized by overexpression of major hematopoietic cell proteins (Gypa, Slc4a1, Lgals5, Hbq1b, Hemgn, Add2, Alas2), anti-inflammatory bioactive lipid mediator arachidonate 15-lipoxygenase (Alox15), CDK-activating dual specificity phosphatase Cdc25B and Neuropeptide Y (Npy) - an important neurotransmitter in skin vascular conductance. Upregulation of the latter genes distinguished VO not only from AO, but also from AVO. Finally, we identified 58 "master" DEGs shared by both genders that were present in SI regardless of its type (AO, VO, and AVO). Of these, "first-tier" proteins included leukemia inhibitory factor (LIF), Depp1 autophagy regulator, adrenomedullin (Adm), growth arrest and DNA-damage-inducible, gamma (Gadd45g) and DNA-damage-inducible transcript 4 (Ddit4). Change in selected gene expression was further confirmed at protein or mRNA level by Western blotting or RT-PCR analyses. In summary, our data revealed several SI-associated diagnostic biomarkers that merit further investigation and, ultimately, validation in the human clinical trial setting.

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