PROXIMAL AND DISTAL EFFECTS OF ARTERIOTOMY IN RAT CAROTIDS: AN EXTENSIVE MICROARRAY ANALYSIS OF GENE EXPRESSION

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Arterial restenosis can follow angioplasty or arterial grafts in humans and experimental animal models. The biology of restenosis is complex and not fully understood. We set up an arteriotomy model of rat carotid injury, in which an asymmetric cut is followed by a fine suture, thus involving the whole thickness of the vascular wall. This model mimics the injury affecting the patient arteries submitted to arterial grafting or endarterectomy, rather than the trauma induced by the commonly applied balloon angioplasty, since it is characterized by an interruption of both the internal and external elastic lamina, considered clinically relevant for the development of arterial stenosis.

We investigated the time course of gene expression in inbred Wistar Kyoto rat injured carotids harvested 4 h, 48 h and 7 days after arteriotomy (n=15 for each group, corresponding to n=3 pools of 5 carotids each for biological and technical replicates) and in contralateral uninjured carotids. Carotids from uninjured rats served as control (n=10). Total RNA extracted from carotid pools was amplified according to Eberwine's method. RNA samples were hybrydized to Affymetrix Rat Genome 230 2.0 Genechips. Microarray data have been validated by reverse transcriptase quantitative Real Time-PCR and by 2D-gel followed by LC/MS/MS. Only array spots with a fold-change of at least 2 were considered for further analysis.

Statistical analysis revealed that the expression of about 1000 genes was significantly affected ($p<0.0001$) 4h after arteriotomy, with a progressive decrease at later times and that about 70% of these genes were up-regulated. The modulated genes have been classified according to their proposed gene ontology biological process. Most genes involved in immune response and intracellular signal transmission were up-regulated early. Others, involved in cytoskeletal organization, protein and RNA turnover and hormonal response, were activated later. Unexpectedly, we detected marked changes of mRNA also in contralateral uninjured carotids, probably induced by the release in the serum of cytokines and growth factors and suggesting compensatory reactions in the contralateral vessels. In particular, we detected in contralateral carotids an up-regulation of mRNA coding for Angiotensin Converting Enzyme and angiotensinogen. Analysis of the temporal sequence of differential gene expression at early stages after injury may give insights into the molecular mechanisms of arterial negative remodeling, providing a basis for the identification of new potential targets for anti-restenosis therapies.