Translational control of the human erythropoietin *via* an upstream open reading frame in cardiac tissue

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Erythropoietin (EPO) is the main hormone that regulates erythropoiesis. Beyond its well-known hematopoietic action, EPO has diverse cellular effects in non-hematopoietic tissues, including cardioprotection. Indeed, in cases of tissue injury the EPO expression increases locally providing a cardioprotective effect supported by numerous experimental data in animal models of ischemia and acute myocardial infarct. Cellular stress activates an integrated stress response, which includes rapid changes in global and gene-specific translation. Translational regulation of specific transcripts mostly occurs at translation initiation and is mediated *via* different *cis*-acting elements present in the mRNA 5' untranslated region (5'UTR); which includes the upstream open reading frames (uORFs). These uORFs modulate translation of the main ORF by decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF. However, in response to abnormal stimuli, they mediate translational derepression of stress-responsive proteins. The 5' leader sequence of the human EPO mRNA has one uORF with 14 codons that is conserved among different species, indicating its potential regulatory role.

In the present work, we aimed to test whether EPO expression is translationally regulated in response to ischemia in cardiac tissue. Reporter constructs containing the normal or mutant EPO 5' leader sequence fused to the Firefly luciferase cistron were tested in H9C2 (rat heart/myocardium myoblasts) and C2C12 (mouse muscle myoblasts) cell lines. Luciferase activity was measured by luminometry assays and normalized to the corresponding mRNA levels quantified by real-time RT-PCR. Results have revealed that the EPO uORF represses translation of the main ORF at about 60-70%, in both cell lines. The results also show the synthesis of EPO protein mainly occurs by reinitiation after uORF translation demonstrating that this uORF suffers low leaky scanning. In addition, our results show that specifically in C2C12 cells, the EPO 3'-

enhancer induces a 4-fold increase in EPO expression, while in H9C2 cells the uORF-mediated translational repression is not affected by the presence of the EPO 3'-enhancer. Nevertheless, in C2C12 cells under chemical ischemia, EPO uORF-mediated translation repression seems to be released. These findings show that cardioprotection effects of EPO might be regulated at the translational level.