# 9. Summary

This study investigated the relationship between COX and chosen AA metabolism-related genes by means of RNA interference. The results are summarized below.

1. In order to prepare a model cell line, four shRNAs against COX-1 and six shRNAs against COX-2 were designed and cloned into expression vectors. The vectors were transfected into Hep2 (cervix carcinoma) cell line and selected with blasticidine. From the set of COX-1-specific shRNA sequences, shRNA1 was a highly effective one (suppressing COX-1 mRNA to ~25.6 % of the basal expression in parent cells). Surprisingly, all COX-2-specific shRNAs enhanced COX-2 mRNA expression. This could be explained by the saturation of exportin 5 that inhibited a natural COX-2 mRNA degradation by miRNA.

2. In addition, synthetic siRNAs were used to inhibit COX-1 mRNA and the down-regulation of ~24 % compared to the basal expression in parent cells was reached. However, siRNA-mediated mRNA suppression is just transient and for that reason it is not possible to reach more significant inhibition by additional procedures, e.g. cell cloning method. Therefore, the strategy using shRNAs was more suitable for further experiments.

3. A few clones with significantly and stable down-regulated COX-1 were prepared by the cell cloning method using the shRNA1-transfected cell line. The COX-1 inhibition was confirmed by real-time PCR on the mRNA level (suppression to ~5.2 % of the basal expression in parent cells), by Western blot on the protein level and by $PGE_2$-ELISA on the functional level.

4. The sets of primers and probes were designed and optimalized for selected genes involved in AA metabolism and the influence of COX-1 inhibition on individual gene expression has been evaluated in chosen COX-1-suppressed clones. The most important findings are summarized below.

a) After the permanent COX-1 inhibition, mPGES-1 was the only isoform from the three PGESs in which mRNA level was significantly changed (down-regulated). Simultaneously, $PGE_2$ synthesis was suppressed. mPGES-1 is believed to be an inducible

enzyme preferentially coupled with COX-2, less often with COX-1. The results obtained in Hep2 cell line brought further evidence that mPGES-1 could pair with COX-1 and be involved in basal $PGE_2$ synthesis. mPGES-2 and cPGES exhibited no transcriptional dependence on COX-1 which indicates that these PGES isoforms may be preferentially coupled with the inducible COX-2 in Hep2 cells. However, this assumption should be further confirmed by mPGES-2 mRNA expression analysis after a specific COX-2 suppression.

b) After the long-term COX-1 suppression, ABCC4 (encoding MRP4 transporter) was significantly up-regulated. This result supports the suggestion that MRP4 has a role in prostanoid transport. Because MRP4 can also mediate the transport of some cytotoxic drugs, this finding could be clinically very important for the combined use of cytostatics and NSAIDs (COX inhibiting drugs) in cancer therapy. Further investigation examining MRP4 substrate specifity to cytostatics and also the influence of individual NSAIDs on ABCC4/MRP4 expression could be helpful for elimination of the use of inappropriate drug (NSAID/cytostatic) combination.

c) After the permanent COX-1 inhibition, TBXAS1 mRNA was significantly enhanced. This result indicates that TBXAS1 could be co-regulated with COX-1 at the mRNA level.

d) The mechanism of the change of mPGES-1 and ABCC4 mRNA levels after COX-1 suppression was specified. It was revealed that COX-1 mRNA inhibition has no influence on mPGES-1 and ABCC4 mRNA stability. These results indicate that mPGES-1 and ABCC4 could share some of the transcriptional regulatory mechanisms with COX-1.

e) The influence of $PGE_2$ on ABCC4 gene expression was evaluated. It was found that $PGE_2$ alone does not affect ABCC4 mRNA level, which is further evidence that the ABCC4 regulation in relation to COX-1 takes place at the transcriptional level.

The following studies could be aimed at:

1. Analysis of the relationship between COX-2 and chosen downstream genes, especially PGESs and ABCC4.
2. Analysis of the influence of NSAIDs on AA metabolism-related genes expression. The comparison of these results with results get after specific COX inhibition.
3. Analysis of the influence of NSAIDs on the expression of ABCC4/MRP4, eventually other transporters (e.g. ABCC5/MRP5).