RNAI-based gene therapy of hepatocellular carcinoma: targeting ABC transporters
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SUMMARY

Hepatocellular carcinoma (HCC) is a primary cancer of the liver, and HCC patients have an average survival of only 5% at 5-year post-diagnosis. This low survival has several identified causes, among which multidrug resistance i.e. resistance to chemotherapeutic treatment. These issues need be addressed in order to improve HCC management in the future.

In this thesis we questioned the role of ABC transporters in HCC, and aimed at developing RNAi-based strategies to compensate their dysregulation. Two strategies were developed to modulate ABC transporter gene expression. The first one exploited endogenous regulation of ABC transporter genes by cellular miRNAs while the second strategy made use of shRNAs and artificial miRNAs for ABCC1 and ABCC2 knock-down.

First of all, the up-regulation of ABC transporter genes and the concomitant global down-regulation of cellular miRNAs in untreated HCC patients were described in Chapter 3. This up-regulation concerns 10 ABC transporters, namely ABCA2, ABCB1, ABCB6, ABCC2, ABCC3, ABCC4, ABCC5, ABCC10, ABCC11 and ABCE1 and suggests that besides their transporter role, ABC transporters may be playing a role in cancer. This work provides the first insights into the importance of ABC transporter genes in untreated HCC patient samples and implies that they might be used as therapeutic targets in the future.

Based on the molecular profiling of HCC, we explored the hypothesis of the regulation of ABC transporter genes by cellular miRNAs. After in silico target predictions, the novel regulations of the expression of five ABC transporter genes (ABCA1, ABCC1, ABCC5, ABCC10, and ABCE1) by cellular miRNAs were predicted and confirmed by luciferase reporter assays in Chapter 3. Regulation of ABCC1, ABCC5, ABCC10, and ABCE1 by cellular miRNAs was a novel finding that has potential relevance for HCC. Regulation of ABC transporter genes by miRNAs was biologically validated in Chapter 4, the cholesterol transporter ABCA1 being regulated by miR-101 and miR-135b, and the multidrug resistance transporter ABCC1 being regulated by miR-199a/b and miR-296. This validation work opens the perspective of a miRNA-based therapeutic approach to modulate ABC transporter gene expression, which could be relevant to decrease clinical multidrug resistance for most ABC transporters, but also to reduce atherosclerosis in the case of ABCA1.

Next, we explored another approach to decrease multidrug resistance transporters in Chapter 5, by using shRNAs targeting ABCC1 and ABCC2. In vitro, the shRNA constructs demonstrated sequence-specific, dose-dependent target knock-down, proper processing into siRNA, and non-activation of the immune response. ABCC2 expression was significantly reduced in wild-type mice upon AAV-mediated shRNA delivery. However, we observed viral dose-dependent hepatotoxicities and corroborated that shRNA overexpression can adversely perturb the RNAi machinery in vivo. Artificial miRNAs that were subsequently developed in order to counteract toxicity, demonstrated target knock-down efficiency similar to that of shRNA while expressing less siRNA molecules.

In conclusion, this research contributed to the development of two RNAi-based strategies to compensate ABC transporter gene dysregulation in HCC. At this moment the RNAi field faces toxicity-related and unanticipated off-targeting challenges that need to be overcome in order to take the technology forward, but once these limitations are fully apprehended, it will undoubtedly lead to significant advances for HCC diagnostics and therapeutics.