MicroRNAs and Noncoding RNAs in Cancer

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1037 Footsteps on the way to hepatocellular carcinoma - the influence of hepatitis B virus infection on histone acetylation, sirtuin activity, chromatin accessibility and the microRNAome

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Background: Virus-host interactions result in altered gene expression profiles in host cell nuclei enabling virus particle production, thus obligatorily involving changes in their epigenomes and microRNAomes. Virus-induced epigenetic changes that contribute to genetic dysregulation and subsequent development of secondary diseases such as hepatocellular carcimoma (HCC) have not yet been understood.
Aim of the study: We investigated the influence of the hepatitis B virus (HBV) on post-translational histone acetylation patterns in the promoter region of oncogenes relevant for the development of HCC and on the microRNAome both in vivo and in vitro.
Methods: We analyzed gene expression of 84 key genes for HCC oncogenesis in a HBV-negative (MMH-D3) and a HBV-positive (HBV-Met) immortalized, non-transformed murine hepatocyte cell line using qPCR. Chromatin immunoprecipitation was carried out to assess histone acetylation levels before and after anti-viral treatment with lamivudine and HBV knock-down experiments using ectopic expression of antiviral siRNA. HAT, HDAC and sirtuin activity was measured using ELISA and luminiscence reporter assays, respectively. Chromatin accessibility was analyzed with a micrococcus nuclease digestion assay. Utilizing next-generation sequencing microRNA libraries were generated from HBV-positive and HBV-negative mice as well as from an HBV-infected human hepatic cell line.
Results: HBV-positive murine hepatocytes showed selective gene deregulation when compared to HBV-negative hepatocytes. HBV-positive cells revealed a global hypoacetylation state at all analyzed loci which was reversible by nucleoside and siRNA anti-viral therapy. While there was no difference in histone acetyltransferase activity, histone deacetylase (class III HDACs/sirtuins) were significantly more active in HBV-Met. Chromatin purified from MMH-D3 cells was better accessible for MNase when compared to HBV-Met chromatin. Human and murine HBV-positive cells showed a significantly altered microRNAome in comparison to HBV-unaffected hepatocytes both in vivo and in vitro.
Conclusions: Reversible hypoacetylation of histones in liver cell nuclei accompanies pre-cancer transregulatory gene expression induced by HBV. These changes are possibly driven by upregulation of class III HDACs/sirtuins and subsequent decreased chromatin accessibility. Furthermore, HBV significantly alters the expression of hepatic microRNAs which possibly promotes the development of HCC.

1038 The miR-200 microRNA family orchestrates hair follicle development by governing migration and proliferation of hair follicle progenitors

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The miR-200 family is highly enriched in mammalian epithelial tissues. Although this miRNA family has attracted escalating interest for their functions in human cancer, their role during epithelial development is not well understood. Using *in* situ hybridization and miR-seq, we determined that miR-200 family expression is further elevated in developing hair follicle progenitors. To interrogate the functions of this miRNA family in hair follicle development, we generated a skin-specific inducible mouse model of the miR-200b cluster (miR-200a, miR-200b and miR-429). Forced expression of miR-200s in the basal epidermis and hair follicle progenitors in these mice leads to multiple defects in hair follicle specification and downward growth, implicating the involvement of this miRNA family in both migration and proliferation. When we examined this miRNA family with both gain- and loss-of-function approaches in primary cultured keratinocytes, we observed concurrent control of proliferation and migration in a cell-autonomous manner. Finally, using a combination of mRNA-seq and Ago2 HITS-CLIP, we have identified high confidence targets for the miR-200 family in the skin. Taken together, our study reveals critical roles for miR-200s and provides molecular insights for hair follicle development.

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1039 Tracing microRNA induced metastasis in cancer cells by magnetic resonance imaging

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Fluorescence application is a huge step for cell tracing especially in the *in vitro* circustances. However, the penetration depth of fluorescence *in vivo* limit its application. Magnetic resonance imaging (MRI) has unlimited penetration depth diction capability and free of ionizing radiation that is ideal for living organism observation. OATP1B3, a transporter protein, can enhance MRI signal by absorbing Gd-EOB-DTPA as contrast medium is used here as a reporter gene for tracing microRNA transfected tumor cells.
We infected HT-1080, a fibrosarcoma cell line with OATP-1B3 by lentivirus. The ovexpression of OATP1B3 was confirmed by western blot and immunofluorescence. Further, the MRI signal was enhanced strongly in OATP1B3 overexpressed cells in *in vitro* and *in vivo*. Afterwards, the mimics or inhibitors of miR-21 and miR-146a, microRNAs that are related to metastasis, are infected into these OATP1B3 transfected HT-1080 cells. As we expected, these two microRNAs could improve cell proliferation by MTT assay. Besides, after treatment of mimic and inhibitor, we observed the invasion behavior enhanced, by transwell analysis. In conclusion, the model is ideal for tracing the microRNA such as miR-21 and miR-146a *in vitro*. Future studies such as observing the invasiveness and metastatic focus of microRNAs infected cells *in vivo* might facilitate the development of further strategy toward cancer treatment.

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1040 Involvement of miRNA-29b in Breast Cancer

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Breast cancer is the most common cancer in women which is the leading cause of cancer death in women. Vast numbers of noncoding RNA molecules have been investigated to get mechanistic insights to breast cancer concerning microRNAs as the modulators of post-transcriptional machinery. miRNA-29b has tumor suppressive properties involved in angiogenesis, proteolysis, extracellular matrix signaling and remodeling and in epithelial mesenchymal transition.

We investigated miRNA-29b levels of 45 breast cancer patients' tumor and adjacent healthy tissues. For this purpose, total RNA was isolated from fresh frozen breast tissues and cDNA was synthesized. Real-time experiments were performed with SYBR Green technology (EXICON) on LightCycler 480 (ROCHE) platform. SNORD48 was used as reference miRNA for relative quantification of miRNA-29b expression.

We observed a significant decrease of miRNA-29b expression in tumor tissues then matching adjacent healthy tissues (p=0.03). Median values for tumor and health tissues were 0.063 and 0.309 respectively.

It is evident also in our study that decrease in miR-29b in mammary tissues confers to malignancy. The results presented here are preliminary findings of our ongoing study.