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Dissertations-Kurzfassung

MicroRNA-21 regulates tumor suppressor Pdcd4 at the post-transcriptional level, and induces invasion and metastasis

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Tumor-suppressor Pdcd4 inhibits transformation and invasion, and is known to be downregulated in cancers. miRNAs, a new class of non-coding RNAs that function as post-transcriptional gene regulators, are increasingly showing to fulfill a profound role in cancer etiology. They can regulate their targets directly by mRNA cleavage or by repressing their translation, depending on the degree of complementarity between the miRNA and the target. The aim of my thesis was to find out whether miRNAs are responsible for the loss of Pdcd4 expression in cancer, and what could be the consequence of such a regulation upon cancer cell invasion, and the metastatic potential. Towards this objective, we first performed a bioinformatics search which revealed a conserved target-site for miR-21 within the Pdcd4-3'-UTR at 228–249 nt. In 10 colorectal cell lines, an inverse correlation of miR-21 and Pdcd4-protein was observed, where cell lines with high endogenous miR-21 expressed low amounts of Pdcd4 protein, whereas cell lines with low amounts of miR-21 showed high amounts of Pdcd4 protein. Transfection of Colo206f-cells, characterized by very low endogenous miR-21 amounts, with miR-21 significantly suppressed a luciferase-reporter containing the Pdcd4-3'- UTR. In contrast, the transfection of RKO cells, characterized by high endogenous miR-21 amounts, with anti-miR-21 increased the activity of the Pdcd4-3'-UTR. This was abolished when a construct mutated at the miR-21/nt228–249 target site was used instead. Anti-miR-21-transfected RKO cells showed an increase of Pdcd4-protein and reduced invasion with no change in Pdcd4 mRNA. Moreover, these cells showed reduced intravasation and lung metastasis in a chicken–embryo–metastasis assay. Tumor weight analysis showed a significant reduction in the primary tumors formed by anti-miR-21-transfected RKO cells. In contrast, overexpression of miR-21 in Colo206f cells reduced Pdcd4-protein amounts and increased the invasive potential of these cells, while Pdcd4-mRNA was unaltered. Additionally, these cells formed significantly larger tumors in the chicken-embryo-tumor formation assay. In corroboration with these results, resected normal/tumor tissues of 22 colorectal cancer patients demonstrated an inverse correlation between miR-21 and Pdcd4-protein, not mRNA. This is the first study to show that Pdcd4 is negatively regulated by miR-21. Furthermore, it is the first report to demonstrate that miR-21 induces three different steps of the metastatic cascade, invasion, intravasation, and metastasis.

As an additional method objective of my thesis, I have established a tissue-ChIP (chromatin immunoprecipitation) protocol for determining the binding of transcription factors to endogenous promoter motifs in resected tumor and normal tissue samples of solid cancer patients. With the resulting protocol, we show a higher binding of c-Jun (a major representative of AP-1 transcription factors) to the proximal AP-1 site located in the region -190/-171 of the u-PAR gene promoter in resected colon cancer tissue as opposed to normal tissues. This protocol can successfully be applied to identify the binding of transcription factors to endogenous promoter motifs *in vivo*, even in solid tissues for which, up to now, no publication has been available. This methodology can significantly add to the elucidation of tissue specific *in vivo* transcriptional mechanisms.