Circulating miR-210 as a novel hypoxia marker in head and neck cancer

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Abstract
A novel technique to detect plasma miR-210 levels was developed to compare against CA-9 hypoxia staining in head and neck cancer (HNSCC). An initial cohort of 11 pancreatic cancer patients and 14 age-matched controls were used as a test set and a second cohort of 11 pancreatic cancer patients and 11 controls were used to validate miR-210. miR-210 was reliably detected and quantified, with a statistically significant 4.0-fold increase in expression in pancreatic cancer patients compared to normal controls (p<0.0004). This difference was confirmed in the validation group (p=0.018). A third cohort of 11 head and neck cancer patients with corresponding CA-9 hypoxia staining was found to demonstrate 3.0x overexpression (p<0.0004). Moreover, a significant correlation between plasma miR-210 expression and CA-9 hypoxia staining was identified. In summary, circulating miR-210 levels were quantified via a novel technique from plasma, and found to be elevated in pancreatic cancer but underexpressed in head and neck cancer. Based upon expression levels and hypoxia correlation with CA-9, miR-210 may potentially serve as a biomarker for HNSCC diagnosis.

Introduction
Although tumor markers greatly improve diagnosis of cancers, the invasive nature of current procedures limits their application. There is therefore a great need to identify novel non-invasive biomarkers for early tumor detection. MicroRNAs are small non-coding transcripts involved in many cellular mechanisms, including tumorigenesis. miR-210 in particular has been shown to be regulated by hypoxia and correlated with adverse clinical outcomes in breast and colorectal cancers and it is a zinc metalloenzyme and associated with hypoxia. In the present study, miR-210 as a diagnostic marker or prognostic indicator for HNSCC was assessed. A novel, non-invasive test was developed that quantifies miR-210 from plasma. miR-210 expression was first quantified in pancreatic cancer, then expanded to head and neck cancer patients. Hypoxia measured by CA-9 staining was also performed for correlation.

Cohort I exhibits 4.0x miR-210 overexpression

Figure 2: 11 pancreatic CA pts compared with 14 age-matched controls. Relative expression calculated by 2(-ΔΔCt value) (4.0x overexpression, ΔΔCt method, triplicate runs, p<0.0004).

Cohort II exhibits 1.6x miR-210 overexpression

Figure 3: 12 pancreatic CA pts compared with 11 age-matched controls. Relative expression calculated by 2(-ΔΔCt value) (1.6x overexpression, ΔΔCt method, duplicate runs, p<0.018).

HNSCC exhibits 3.0x miR-210 underexpression

Figure 4: 38 HNSCC pts compared with 11 age-matched controls. Relative expression calculated by 2(-ΔΔCt value) (3.0x underexpression, ΔΔCt method, duplicate runs, p<0.0004).

Figure 5: (1) Primary miRNA transcripts. (2) Hairpin-like structure signals digestion to produce precursor miRNA (Pre-miRNA). (3) Nuclear export of Pre-miRNAs. (4) Cleaving of pre-miRNA. (5) The miRNA/RISC complex recognizes and binds to complementary translation by binding miRNA 3’ regions.

Discussion
Tissue-based diagnosis remains invasive and time-intensive. In this study, a novel noninvasive protocol consuming a minimum of plasma sample is developed. It was found that miR-210 was significantly overexpressed in pancreatic cancer, chosen because of its known hypoxic microenvironment. Interestingly, HNSCC miR-210 expression correlated with CA-9 hypoxia staining (p=0.05), but was underexpressed relative to healthy controls. In the majority of cancer systems studied, miR-210 appears to be upregulated. One exception is ovarian cancer, which loses the gene locus encoding miR-210. Certain other miRNAs exhibit downregulation with low oxygen tension, including miR-15b, miR-16, miR-122, miR-30b, and miR-491. It is unclear whether this is the result of cell cycle arrest, or if these miRNAs are specific targets of hypoxia-inducible factor (HIF). A major regulator protein that directs hypoxia-related mechanistic studies clarifying miR-210 function are needed to further understand this unique finding. However, the correspondence of miR-210 with hypoxia intensity remains a significant finding. Given that degree of hypoxia is related to a poor prognosis, a novel miR-210 expression as quantified by this clinically applicable, non-invasive protocol shows promise as a novel biomarker for HNSCC diagnosis.