A Study of Mir-210 Affecting Tumor Growth in a Knockout Mouse Model

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Foremost, I would like to express my sincere gratitude to my advisor Prof. Yan Zeng for the continuous support of my study and growing, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this report. I could not have imagined having a better advisor and mentor for my Ph.D study.

Besides my advisor, I would like to thank Xiaoxiao Zhang, for her dedicated guidance, patience and encouragement.

microRNAs (miRNAs) are a class of small non-coding RNAs found in many multicellular organisms. They are approximately 22 nucleotides long and primarily function to regulate gene expression by reducing the stability and translation of target mRNAs. Over the past decade, microRNAs have emerged as key regulators of many physiological and pathological processes. So far, more than 1,000 human miRNAs have been identified that may be involved in cell proliferation, differentiation, and viability. Furthermore, emerging data implicates miRNAs' roles in the development of many diseases such as cardiovascular diseases, inflammatory diseases, and various types of cancer. It has been suggested that miR-210 overexpression is associated with tumor growth in a broad spectrum of cancer types, such as pancreatic cancer, and breast cancer. Notably, a deletion of miR-210 was reported in human ovarian cancer, so miR-210 is not simply overexpressed in all the cancer types.

We have generated a miR-210 knockout mouse model in the lab (Figure 1) to study the functions of miR-210 in mammalian systems. For this project, first I have produced and identified wildtype (WT) and homozygous miR-210 knockout (KO) mice through mating and genotyping. Then the growth and overall behaviors of the mice was monitored over time. We will also sacrifice the animals and analyze gene expression in different tissues. This will ascertain whether the reported miR-210 targets are truly regulated by miR-210 in this in vivo system as well as identify potential, new miR-210 targets. For these new targets, we will perform reporter assays to confirm their interaction with miR-210 using cell cultures.

1. Generation of miR-210 WT and KO mice.

We have bred heterozygous male and female mice to produce WT, heterozygous KO, and homozygous KO mice. For genotyping, <0.5 cm mouse tail is removed, suspended in a solution containing SDS and proteinase K at 55oC overnight. After phenol/chloroform extraction, genomic DNA is precipitated with ethanol and dissolved in water. Then PCR with specific primers was run to identify WT, heterozygous and homozygous miR-210 KO animals (Figure 1). If miR-210 loss does not affect the overall viability, we expect that the above genotypes will be produced at a 1:2:1 ratio, and male:female ratio is 1:1.

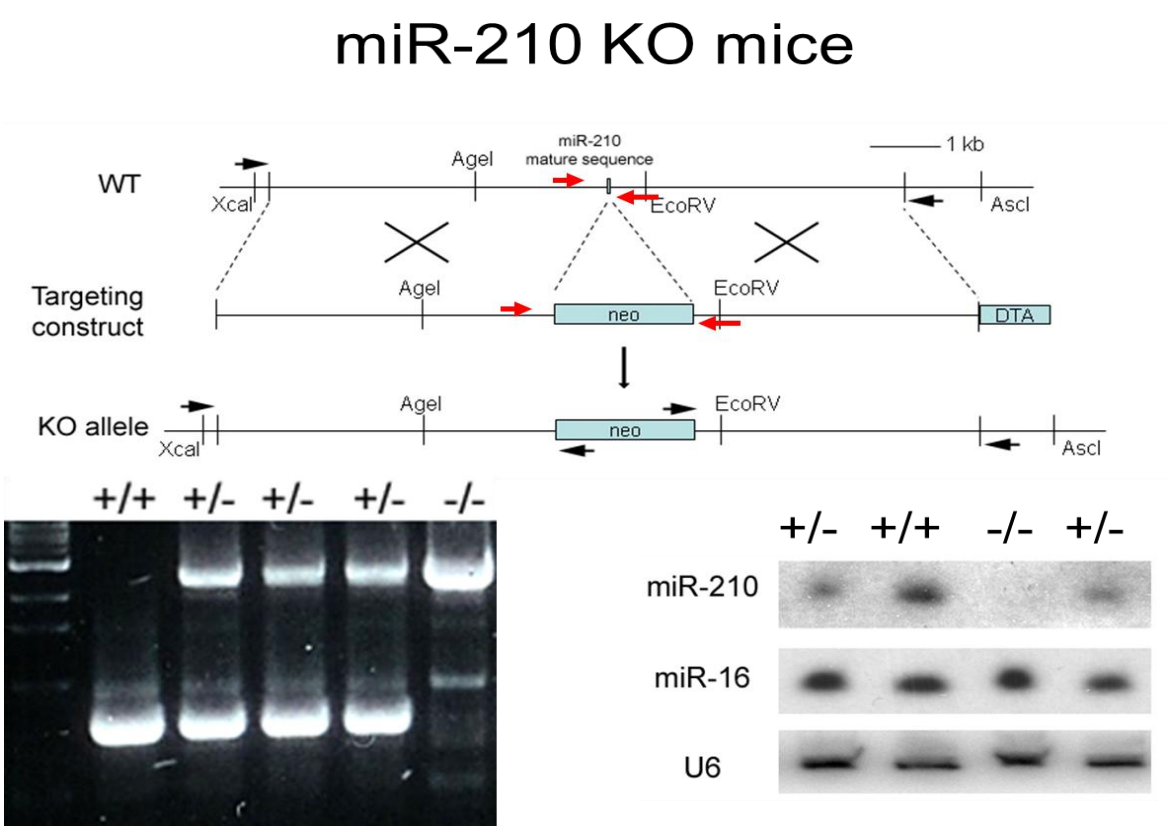

Figure 1. Schematics of the targeting strategy and the genotypes identified by gel electrophoresis. Only the mature miR-210 sequence was deleted and replaced by a neomycin resistance gene cassette (designated as "neo"). DTA: diphtheria toxin A gene for negative selection. Horizontal arrows (not drawn in scale) symbolize the primers used for PCR genotyping. The genotypes of the mice are identified by gel electrophoresis.

2. Monitor the growth of the mice over time.

The mice were inspected and weighed once every 3-4 days to examine the growth difference between WT and homozygous KO littermates. The weight on the 20±5day and 50±5day are compared within male and female group. We found that on the 20±5day after born, the KO group has slightly lower body weight compared to mice in WT group for both male and female, These results suggest that WT mice grow slightly faster than the KO mice. However, due to the limited number of mice being examined, it is difficult to see clearly if the body weight difference between two groups is statistically significant. To observe such subtle difference in the two types of mice, we might need more defined experimental protocols.

Male Genotype	number of mice weighed	mean weight on 20±5day	Ratio to +/+	mean weight on 50±5day	Ratio to +/+
+/+	6	8.82	100%	18.15	100%
-/-	10	8.51	96.5%	18.27	~100%
Female Genotype	number of mice weighed	mean weight on 50±5day	Percentage of -/-	mean weight on 100±5day	
+/+	7	26.86	100%	34.1	100%
-/-	7	24.51	91.2%	33.74	~100%

Table 1. The body weight of litters were measured every 3-4 days. The weight on the 20±5day and 50±5day are compared within male and female group.

3. Study the tumorigenesis in miR-210 KO mouse model.

It is known that miR-210 overexpression has a key role in a variety of cancer. To examine the miR-210 function related to tumorigenesis *in vitro*, miR-210 KO mice were given tumor cells to examine the tumor growth. First, mice carrying mir-210 alleles (+/+ genotype) and mir-210 KO mice (-/- genotype) were generated by crosses of wild type and mir-210 mice. Groups of mice with +/+, -/- backgrounds, each consisting of about 25 animals (20 weeks old), were given a single subcutaneous injection of tumor cell (400-500mg). Approximately two weeks after injection, these mice were killed once the first mouse was dead, and the tissues were examined. Tumors were weighed and fixed in formalin for further study. The tumor size difference is shown in Fig. 3. There is no significant difference of tumor size between WT and KO mice ($p=0.958$). However, as shown in Fig. 1, there was a distinct difference in survival rate of the three genotypes of mice. The death rate for WT mice was 12%; whereas it was 3.4% for KO genotype. On the other hand, the rate of metastasis was 7% and 8% for WT and KO genotype, respectively. The metastasis was found in the body as small black tumor dots. These results suggest that high expression of miR-210 might predict poor survival rate in mice but not necessarily larger size of the primary tumor. And more trials of experiments need to be undertaken to further address the relationship.

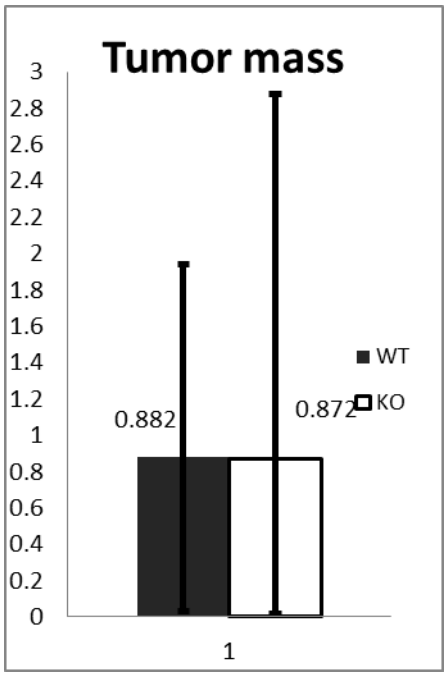

Fig 3. The tumor mass is compared between WT and KO mice that were injected with cancer cells. The mean mass for WT is 0.882g, for KO is 0.872g. The p-value is 0.958, which indicates that there is no significant difference of tumor size observed between WT and KO mice in this study.

mice genotype	Total number of mice examined	number of mice dead when harvesting	number of mice with metastasis
+/+	25	3 (12%)	2(8%)
-/-	29	1(3.4%)	2(7%)

Table 2. Survival rate and metastasis rate is compared between WT and KO mice.

Reflection

The project I did was to study mirRNA effecting tumor growth in a knockout mouse model. During my research experience, I learned a variety of technical skills in molecular biology, such as genotyping, DNA extraction, protein purification and western blot. In addition, I learned using

animal model to study a biological problem *in vivo*. These hand-on research experiences would help me to conduct biological researches in the future. During my time working in the lab, I have learned a lot more from my faculty mentor, Dr. Yan Zeng. I frequently interacted with him and discussed not only my research work, but also my career goals in the long term. I learned from him that scientific research can be both exciting and dull. To be a scientist, it is important to be persistent and optimistic even when one gets stuck (and it happens a lot!). Furthermore, my UROP experience gave me a great chance to learn cutting-edge researches going on in the university and other institutes through attending seminars and reading scientific journal articles. AT the end of my project, I wrote my UROP report the guidance by my faculty mentor. I learned different methods to collect and analyze data, and I also practiced writing a research report in a scientific way. I gained analytical and critical thinking skills, which are very important for my future career as a scientific researcher.