The Role of Micro RNA 3161 in Melanoma Cell Lines

by Bar Laub

Melanoma is a type of cancer that stems from Melanocytes.[1] Cancer is a broad classification of related diseases, that can be further divided into subcategories: carcinomas, sarcomas, and leukemias or lymphomas.[2] The vast majority of the cancer diagnoses fall under the carcinomas.[2] Carcinomas arise from epithelial cells.[2] In humans, there is a low probability to develop cancer that arises from muscle tissue, cartilage, bone tissue, and fibrous tissue. About 8% of the cancers in humans are leukemias and lymphomas.[2] These cancers can arise from the immune system cells.[2] Cancer stem from dysregulation of the cell cycle.[3] The cell cycle is a term associated with cell growth, DNA synthesis, and division of one mother cell into two daughter cells.[2] In Eukaryotes there are main four stages for the cell cycles: the G1 phase, the S phase, the G2 phase, and the M phase.[2] Cell division duration of human cells in cell culture takes generally twenty four hours.[2, 4] 95% of the cell cycle duration is spent in the G1, S and G2 phases meanwhile the M phase where the mitosis and cytokinesis occurs, it lasts about an hour.[2] The stage when a cell is metabolically active but do not proliferate (i.e. divide) is called G0 phase.[2] With flow cytometry and a fluorescent dye that binds to the DNA, one can estimate the different phases in the cell cycle.[4, 5] The emitted fluorescent light is proportional to the amount of DNA in the cell.[4, 5] During the S phase DNA is synthesized and when the cell reaches the point of double of the DNA concentration that is found in a cell at the G1/G0 phases.[4] The cell enters the G2/M phase when the DNA synthesis is completed.[4] The cell cycle is regulated by intrinsic and extrinsic factors and processes.[2] The main check point between the G1 phase and the S phase is the RB protein (Retinoblastoma protein).[4] There are other check points to reduce errors. [4] These check points prevent the progression of the cell cycle when DNA damage is detected or if the chromosomes did not attach correctly.[4] The check pints are controlled largely by cyclins dependent kinases. [4] Protein kinases and phosphatases are able to activate and deactivate a large proportion (estimated to be

30%) by the addition or the removal of a phosphate group.[6] Kinases transfer the gamma phosphate from adenosine triphosphate (ATP) or guanosine triphosphate (GTP) on to a protein.[6] This results in a structural conformational change to the target protein.[6] The cell cycle dysregulation leads to an increase in proliferation; hence increasing the chance for errors and further accumulation of mutations that could lead to differentiation, and late stages to migration.[7] This process harms other body tissue and can result in many cases to death.[3, 8] When a cell undergoes a malignant transformation it becomes cancerous.[3] There are four main stages in the differentiation of cancer.[3] The first is the accumulation of one or multiple mutations that increase its likelihood of division of the mother cell.[3] The second stage is also known as hyperplasia is when the cells accumulate mutations that lead them to divide excessively often.[3] The third stage is when the cells began to differentiate and appear abnormal in contrast to healthy cells.[3] The fourth stage is when the cancer tissue is highly abnormal physiologically and visually.[3] The cancer tissue could either stay contained at the tissue where it emerged or migrate throughout the blood system or through the lymphatic system and establish metastasis throughout the body.[3] Metastasis are defined as the invasion and migration of cancer out of the tissue of origin.[9] The consensus in the last 50 years suggests that the vast majority of cancer deaths are caused by metastasis.[9] Cancer cells that emerged from epithelial cells could differentiate into cells with mesenchymal features.[9] These changes lead eventually to dysregulation in the cell to cell and cell to the extracellular matrix adhesion mechanisms.[9] Thus, it results in an increase in the likelihood to spread to distances organs.[9]
Cancer accumulates mutations in an increasing manner throughout its progression hence; it is subjected to natural selection[10] Beneficial mutations to the tumor such as increasing of DNA repair mechanisms, inhibitions of apoptosis, drug resistance abilities result in survival of the tumor.[10] Numerous micro RNAs are involved in the drug resistance of cancer tumors.[10] Some cancer tumors can develop multi drug resistance that is commonly facilitate by ATP (Adenosine triphosphate) binding cassette transporter.[10] The transporters pump out the chemotherapeutics from the cytoplasm to the extracellular space.[10] Melanoma occurs when melanocytes undergo malignant transformation. [11] Throughout 2019 in the U.S. alone 96,480 new cases of Melanoma

are estimated to be diagnosed.[12] Melanoma accounts for most of the skin cancers related deaths.[12, 13] The chances to survive five years with a stage four melanoma is 10% in contrast to 97% chances to survive five years with melanoma at stage zero.[14] Melanoma accounts to the fifth most common cancer in men and accounts to 5% of all cancers in men.[14] Meanwhile melanoma is the seventh most common cancer in women and accounts to 4% of all cancer in women.[14] Initially melanoma grow horizontally in the epidermis.[14] In later stages it began to grow vertically into the dermis and may differentiate further into metastasis.[14] Malignant transformation is caused either by random mutation, DNA damage from radiation or cancerogenic chemical. [3] In 50% of melanoma cases, deregulation of the BRAF gene is present. [15] BRAF is a serine/threonine protein kinase that regulates the MAPK/ERK pathway. [15] Dysregulation in the MAPK/ERK pathway affects the cell cycle clock, which could potentially lead to cancer. [15] This pathway dysregulation might be influenced by the micro RNA 3161.

Micro RNAs (miRs) are a large class of small non coding RNAs that regulate approximately 60% of human genes.[16] micro RNAs are short messenger RNA strands approximately 22 nucleotides long.[16, 17] Oncogenic micro RNAs are up regulated in cancer meanwhile micro RNAs that down regulated oncoproteins are down regulated.[18] Micro RNAs attaches to the micro RNA response elements (MREs) regions of the messenger RNA. Many are found at the 3' untranslated region (3'utr).[16, 17] micro RNA starts as a messenger RNA molecule that folds and binds to itself to form a hairpin loop, known as pri-micro RNA.[16, 17] It is cleaved by the Drosha ribonuclease iii to form pre-micro RNA and then exported outside of the nucleus via Exportin 5 transport protein.[16, 17] A further processing of the pre- micro RNA is done by the Dicer ribonuclease iii to form the miRNA:miRNA* duplex by removal of the hairpin loop.[16, 17] This complex then attaches to the RNA induced silencing complex (RISC) which composed out of numerous proteins.[17] The Piwi domain of the Argonaute protein will cleave the passenger RNA stand the guide RNA stand meanwhile will attach to the Paz domain.[48] The Paz domain is the domain where the mature micro RNA binds to guide the RISC complex to the messenger RNA molecule of the inhibited gene.[19] Nucleation is the attachment of the seed region of the micro RNA to the target messenger RNA.[17] Following the messenger

RNA attachment to the seed region of the micro RNA at the RISC complex will only cleave the messenger RNA that will be further degraded or inhibit the attachment of the ribosome's subunits hence lower inhibition of the translation.[17] The seed region allows one micro RNA to regulate multiple genes because approximately seven nucleotides long match out of the about 22 nucleotides long micro RNA is required.[17] The difference between those mechanisms is that in one the RISC complex rapidly disables a large amount of messenger RNA (RNA cleavage mechanism) while the second does not(ribosome subunits attachments inhibition).[17] By binding to the messenger RNA, the micro RNA represses and prevents translation.[17, 20] For instance, overexpression in micro RNA 1296 in breast cancer was observed to suppress cancer progression.[21] The specific micro RNA 3161 has not been thoroughly studied yet (March 2020).

The study was designed in such a way to find if the lead, down regulation of the micro RNA 3161 in multiple cancer cell lines, has the desired pro apoptotic effects and if further investigation is required. I transfected the human melanoma cell lines with the micro RNA 3161. Then, I preformed colony formation assay to observe if an elevation in the micro RNA 3161 caused a decrease in the number of colonies. To observe the effects, I performed a cell cycle fluorescent flow cytometry assay and cell death fluorescent flow cytometry assay. These experiments could backup or disprove the hypothesis that micro RNA 3161 drive apoptosis in melanoma cell lines.

I then used the guiding region (seed region) of the micro RNA 3161 to find, bioinformatically, of regulatory proteins targets. I tested numerous protein targets via western blot and tried to correlate the up regulation of the micro RNA 3161 to down regulation of oncogenes or the up regulation of pro apoptotic.

My superior and choose protein targets based on if they support the study hypothesis and if we had primary antibody in the laboratory. I tested if the protein targets were up or down regulated via western blot. First, I transfected the cells with micro RNA 3161 and grew them for 48 hours before I extracted the proteins and quantified the extract concentration. Then I prepared a 10% acrylamide gel to and heat shocked the proteins samples. To normalize the control sample and the micro RNA 3161 transfected cells I used GAPDH.

After numerous tries I found one protein target that was dysregulated in compare to the control.

The research results are confidential. Nonetheless I preformed the experiments and supported my supervisor work. I learned how to design a study, time management and how to preform these experiments and assays. Micro RNA 3161 did had a significant effect on melanoma cell lines and with the current research on RNA vaccines it is possible that a micro RNA based cancer therapeutic could be developed in the future.

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