Two-Column Template for LACCEI Conference Proceedings Based on IEEE Format (Title)

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Abstract

For all cancers, increased ribosome biogenesis is a critical requirement and RIO kinases play a critical role in maintaining processing efficiency. RioK1 is important because it is required for cytoplasmic maturation of the small(40S) subunit. RioK1 is also important to other cancer promoting processes, such as proliferation and migration. In functions other than ribosome biogenesis, RioK1 is used as an adaptor protein of PRMT5 (protein arginine methyltransferase 5) and recruits nucleolin for symmetrical demethylation. In relation to cancer, however, specific roles, as well as structure for the RioK1-PRMT5-nucleolin complex is still unknown. Overall, this research will investigate the effects of RIOK1 knockdown and overexpression on cancer-supporting processes such as proliferation, migration, and invasion. The goal of this specific project was as such: what is the ideal molar ratio of mixing RioK-1 and PRMT5, that results in the best, most homogeneous, complex being formed? After determining this ratio, X-ray crystallography will be used on the RioK1-PRMT5-nucleolin complex and the structure will be determined. The methodology used was as follows: first, Riok1 and PRMT5 from Chaetomium Thermophilum were expressed in E. coli bacteria and then purified. Afterwards, RioK1 and PRMT5 were mixed in various molar ratios and then analyzed on native PAGE gel. Initially, RioK1 and PRMT5 were both varied, but then RioK1 was kept at a constant ratio while PRMT5 was varied. Our results showed that the ideal molar ratio for mixing RioK1 and PRMT5 was a 1:4(Riok1:PRMT5) molar ratio. These results show that the 1:4 molar ratio is ideal, but it is not known if the ratio is defined on PRMT5 being in excess of RioK1—another test must be done in which PRMT5 is held constant while RioK1 is varied. The results of this project have positively contributed to the overall research goal: the evaluation of RioK1 as a potential target and potentially providing new strategies for anti-cancer therapy.

INTRODUCTION

CANCER IS A DANGEROUS DISEASE THAT AFFECTS MANY PEOPLE IN THE WORLD TODAY. CANCER IS THE TERM GIVEN TO A COLLECTION OF RELATED DISEASES. IN ALL TYPES OF CANCER, THE BODIES CELLS BEGIN DIVIDING WITHOUT STOPPING AND SPREAD INTO SURROUNDING TISSUES.[1] CANCER CELLS DIFFER FROM NORMAL CELLS IN THE FACT THAT THEY ARE LESS SPECIALIZED THAN NORMAL CELLS. NORMAL CELLS USUALLY MATURE INTO DISTINCT CELL TYPES WITH SPECIFIC FUNCTIONS, WHILE CANCER CELLS DO NOT. CANCER CELLS, DUE TO THIS UNSPECIFIED FUNCTIONALITY, CAN IGNORE SIGNALS THAT NORMALLY SIGNAL THE END OF CELL DIVISION. IN THOSE REGARDS, OUR STUDY WORKS ON OVARIAN CANCER CELLS. OVARIAN CANCER OCCURS IN THE FEMALE REPRODUCTIVE GLAND, THE OVARIES—FIGURE 1 SHOWS THE DIFFERENCE BETWEEN A NORMAL OVARY AND ONE AFFECTED BY CANCER.[2]

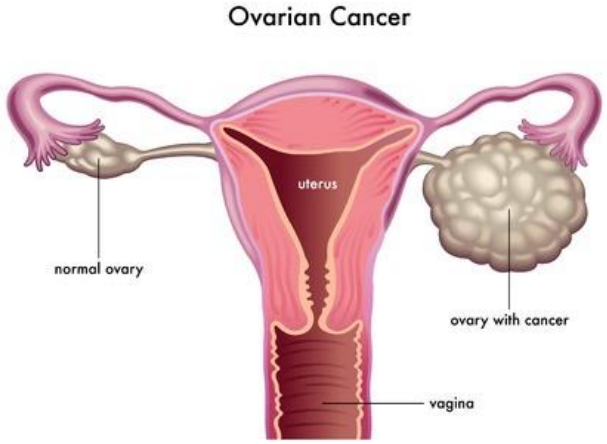

FIGURE 1. DIAGRAM VISUALIZING OVARIAN CANCER

1 IN 75 WOMEN ARE AFFECTED BY OVARIAN CANCER AND 1 IN 100 OF THOSE AFFECTED, HAVE A CHANCE OF DYING FROM THE DISEASE; TO COMBAT THIS DISEASE, THIS RESEARCH IS IMPERATIVE.

RIO-KINASE 1(RIOK1), PROTEIN ARGININE METHYLTRANSFERASE 5(PRMT5) AND NUCLEOLIN ARE THREE PROTEINS THAT FORM A COMPLEX WHICH RELATES TO CANCER. THIS COMPLEX IS FOUND IN NORMAL CELLS AS WELL, BUT IN CANCER CELLS IT HAS BEEN FOUND THAT RIOK1 IS DIRECTLY INVOLVED IN CANCER SUPPORTIVE PROCESSES, ALTHOUGH THE EXACT ROLE IS UNCLEAR. IN ORDER UNDERSTAND THE ROLE RIOK1 PLAYS, THIS PROJECT FIRST FOCUSES ON DETERMINING THE STRUCTURE OF THE RIOK1-PRMT5-NUCLEOLIN COMPLEX AND FROM THERE, THE EFFECTS OF RIOK1 KNOCKDOWN AND OVER EXPRESSION ON CANCER SUPPORTIVE PROCESSES SUCH AS PROLIFERATION, MIGRATION AND RIBOSOME BIOGENESIS WILL BE STUDIED.

RIBOSOMES ARE AN IMPORTANT REQUIREMENT FOR PROTEIN SYNTHESIS IN ALL KINGDOMS OF LIFE. BOTH PROKARYOTIC AND EUKARYOTIC RIBOSOMES CONTAIN TWO SUBUNITS, THE SMALL 40S SUBUNIT AND THE LARGE 60S SUBUNIT. THE 40S SMALL SUBUNIT (SSU) CONSISTS OF ONE RRNA (18S) AND 33 PROTEINS AND THE 60S LARGE SUBUNIT (LSU) CONSISTS OF

three rRNA (25S, 5.8S and 5S) and 46 proteins.[3] Both the large and small subunits of ribosomes undergo assembly processes, in which maturation occurs (Figure 2).

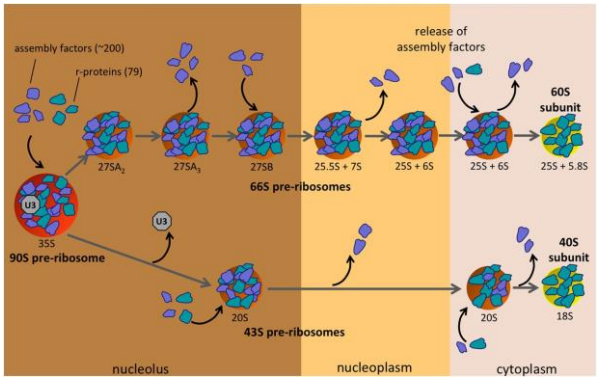

Figure 2. Diagram showing the maturation stages of eukaryotic ribosomes[4]

The diagram shows that ribosome biogenesis occurs in multiple location throughout the cell. As can be seen in the diagram, the 43S pre-ribosomes are exported out of the nucleus into the cytoplasm, before maturation into the 40S subunit.

Protein kinases of the RIO family are required for cytoplasmic pre-40S maturation, particularly both RioK1 and RioK2 control the recycling of transacting factors that associated with late pre-40S ribosomes. RioK2 is usually released with several small subunit biogenesis factors before RIoK1 is released. RioK1 is important in the process of passing pre-mature small subunit particles through the last maturation step: where the 20S ribosomal subunit is cleaved and the mature 40S subunit is produced (figure 2).

RioK1 also functions as an adaptor protein to recruit nucleolin to PRMT5 for methylation. Protein methylation is considered as an important post-translational modification which regulates protein functions in aspects such as catalytic activity, localization, and stability.[5] Arginine methylation is becoming an important post-translational modification for cytoplasmic and nuclear proteins.[5,6] Nine PRMTs have been identified in humans and are classified as two different types based on their specific methylated product. Formation of mono-methylarginines are catalyzed by both type I and type II PRMTs, while the formation of symmetric dimethylarginines is only catalyzed by type II PRMTs.[5] PRMT5 is a major member of the type II PRMTs and catalyzed the mono and symmetric methylation of arginine residues of a wide range of proteins which play critical roles in various cellular processes. This includes RNA splicing, translational regulation, DNA repairing, signal transduction, cell proliferation and stem cell, primordial germ cell differentiation.[5] PRMT5 directly interact with commonly misregulated or mutated proteins in cancer and it is indicated that PRMT5 plays an important role in cancer as a potential oncogene. PRMT5 is overexpressed in multiple cancers including ovarian cancer. For example, in epithelial ovarian cancer, the survival level has been found to be negatively correlated to PRMT5 expression level.[7]

Nucleolin is a universally expressed protein that is localized in both the cytoplasm and nucleolus. It plays essential roles in many pathways including ribosome biogenesis, maturation, and cell proliferation.[8] The connection between nucleolin and PRMT5 is further supported because AS1411, a nucleolin-targeting quadraplex-forming oligonucleotide aptamer, alters the subcellular localization of the PRMT5-nucleolin complex.[9]

The project specific goal is as such: what is the ideal molar ratio of mixing RioK-1 and PRMT5, that results in the best, most homogeneous, complex being formed?

Methods

Protein purification

Chaetomium Thermophilum PRMT5 and Rio-K 1 were expressed in E. coli bacteria. The plasmids used to express these proteins bear a gene that allows for kanamycin resistance. The cells were screened for kanamycin resistance to ensure they contain the protein expressing plasmid. Cells were then grown to an OD of 0.6-0.8 at 37◦C and then 1 mM IPTG was added and the cells were placed in 18°C to express protein for 14-16 hours. The cells are then harvested using Tris-HCl pH 8.0 lysis buffer (100mM Tris, NaCl 300 mM, 2mg/mL RNAase A and 10 ug/mL DNase 1). Buckbuster (0.1-0.5) X was also added, as well as 0.2% BME and the cells were lysed for 45 minutes. The lysate was centrifuged at 20,000 RPM, and the supernatant was then passed over two His-trap columns, a Mono Q-column and a size exclusion column in order purify the PRMT5 and Rio-K1 separately.

Native PAGE gel

PRMT5 and RioK-1 were mixed in various molar ratios buffer containing 200 mM NaCl. 100 mM Tris pH 8.0, and 0.2% BME, incubated for 30 minutes and analyzed on Native PAGE gel. A protein molecular weight ladder used for SDS-PAGE analysis was used due to there not being a native PAGE protein marker.

Results:

Figure 3 shows the results of the Native Page gel and table 1 shows the molar ratio of RioK-1 to PRMT5 mixed together. The ideal ratio for mixing RioK-1 and PRMT5 was found to be a 1:4 molar ratio.

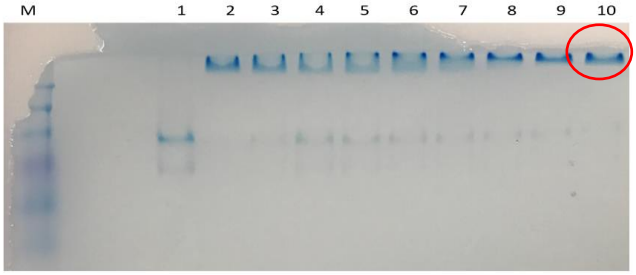

Figure 3. Native PAGE gel results

Well #	Ct-Rio1-molar ratio	Ct-PRMT5-molar ratio
M-Marker	0	0
1	1	0
2	0	1
3	2	1
4	1	1
5	1	1.5
6	1	2
7	1	2.5
8	1	3
9	1	3.5
10	1	4

Table 1. Molar ratios of mixing Ct-Rio-K 1 and Ct-PRMT5

Discussion

In trying to determine the structure of the RioK-1-PRMT5-Nucleolin complex, the ideal molar amount for mixing had to be established. Initially, due the ratio not being ideal, upon mixing RioK-1 and PRMT5, there was still two bands present. For example, in lane 3 of figure 3, when a RioK-1 to PRMT5 molar ratio of 2:1 was used, two distinct bands resulted. However, from lane 4 and onwards, as RioK-1 was kept at a constant molar ratio of 1 and PRMT5 was varied, the bands gradually became one. It is important to note that as the bands became one, they moved upwards on the gel—indicating a bigger size. When two proteins form a complex, it is expected that their size together will be bigger than the two proteins separately. Gel electrophoresis works on the principle that proteins separate by size, with heavier proteins being near the top of the gel while lighter proteins will be near the bottom. As the ratio became more ideal, the bands moved closer to the top of the gel which indicates that a 1:4 Rio-K1: PRMT5 ratio is ideal, which can be seen in lane 10 on figure 3.

For future research, another study could be done in which [RioK-1] is varied while [PRMT5] is held constant. This would be done to verify if the discovered ideal molar ratio of 1:4 is dependent on which protein is in excess of the other, or if only the ratio is important. Construct building of the RioK-1, PRMT5 and nucleolin also needs to be done as well. Lastly, the screening of various drugs on RioK1-PRMT5-nucleolin complex will be done and their effects will be studied.

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