Identification of the key node in the expression network of BLM, EXO1, and DNA2 in Homologous Repair mediated DNA damage response

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Abstract

DNA2, EXO1, and BLM are known to be a part of the end resection rate limiting step of the Homologous Recombination (HR) DNA damage repair (DDR) pathway. Inhibition of these three proteins would in effect inhibit the HR repair pathway. In cancer cells where the HR repair pathway is hyperactivated there is increased risk of metastasis, decreased patient survival rates and chemotherapy resistance. Inhibition of this pathway could lead to a decrease of these side-effects. In this experiment, I tried to establish cell lines that overexpress the DNA2, BLM, and EXO1 proteins in order to do a transcriptome analysis on them and be able to identify common upregulated genes that can be targeted using drugs in order to reverse the chemotherapy resistance in tumors that have uncontrolled HR response. Due to time limitations and the need for protocol troubleshooting, I was not able to finish establishing the cell lines.

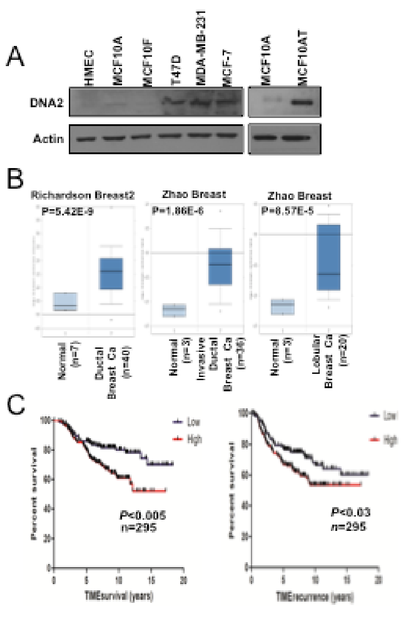

Figure 1. A, Cell lysates from indicated cancer cell lines were subjected to western blot. B, Box plots showing DNA2 mRNA expression analyses. The mean expression values of DNA2 are shown for each group. C, Kaplan-Meier survival curves are shown for overall survival time (left) and time to recurrence (right) stratified by expression levels of DNA2.[9]

Introduction

The human genome is continually exposed to mutagenic stress caused by many physiological processes. One cause is DNA lesions due to mismatches during DNA replication, DNA strand breaks caused by abortive topoisomerase activity, hydrolytic reactions and non-enzymatic methylation. Other causes may include reactive oxygen and nitrogen compounds produced in cellular respiration and inflammation, respectively. Cells have evolved mechanisms to correct the DNA breaks caused by this stress. These DNA damage repair mechanisms include Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ)[1-4]. However, mutations in the DNA repair genes may cause DNA repair pathway deregulation, leading to the development of tumors and increased activity of the DDR mechanisms[5]. Increased DDR in tumors may result in resistance to chemotherapeutic drugs that are DNA damaging agents since the toxic DNA lesions will be repaired with more ease than in cells that do not overexpress DDR proteins. DNA repair inhibitors, however, can exploit tumor-specific defects in checkpoint signaling and DNA repair to amplify toxic replication-associated DNA lesions that directly result in cell death and would therefore be highly effective at killing tumors [6].

Some anticancer drugs function by making DSB which may be repaired by Non-Homologous End Joining (NHEJ) or Homologous Recombination (HR). Studies have shown that HR plays a more prevalent role in the repair of DSB associated with replication forks[7]. The HR pathway is initiated by nucleolytic excision of the DSB in the 5'-3' direction by MRE11-RAD50-NBS1 (MRN) complex[5], this first step is known as DNA end resection. We believe that if we inhibit the rate limiting end resection step of HR-mediated DNA repair, we will be able to enhance cancer therapies in tumors that use this pathway to maintain the genomic stability it needs to survive.

In previous studies of DSB end resection, five factors were associated with this process; MRN complex CTLP, BLM, DNA2, EXO1. These studies demonstrated that the MRN complex and Ctlp are important only in the initiation of resection; and that EXO1 and DNA2, two nucleases, have redundant functions in DSB end resection[8]. If we desire to inhibit the end resection step, the redundant functions of EXO1 and DNA2 could be a limiting factor because they would require a different drug to treat each protein. This factor should be considered in the planning of any experiment oriented towards inhibiting the end resection step since the most beneficial treatment is the one that has the patient exposed to the least amount of drugs.

Hypothesis

By using a transcriptome assay on cell lines that overexpress BLM, EXO1, and DNA2 we will be able to identify the key node in the expression network of these three proteins. With further experiments we will target this key node with drugs in order to inhibit the end resection step of HR and diminish the drug resistance that is confered by this DDR pathway to malignant cells.

Methods

Cell Culture

10A cells were maintained in 10A medium at 37° C with 5% CO_2. 293t cells were maintained in DMEM supplemented with 10% fetal bovine serum at 37° C with 5% CO_2.

Plasmid Amplification

Plasmids were amplified using recombinant E.coli and isolated using GenElute™ Plasmid Miniprep Kit.

Transfection

We transfected 293t and 293ft cells using lipofectamine 2000, MEM medium desired plasmid in 100mm dishes. After 48 hours western blot was performed.

Immunoblotting

Cells were collected and lysed using urea lysis buffer. Equal amounts of protein were separated on Tris-glycine SDS-polyacrilamide gels and transferred to PVDF membranes. The blots were probed with antibodies against BLM (H-300) and EXO1 (SPM394) purchased from Santa Cruz Biotechnology; DNA2 (ab96488) purchased from abcam; and V5 purchased from invitrogen.

Lentiviral Packaging and Transduction

Lentivirus production was done following the protocol in the ViraPower™ Lentiviral Expression System Manual. After lentivirus was packaged we proceeded to infect 10A cells previously seeded in a six well plate. After 24 hours cells were then selected using drugs.

Results

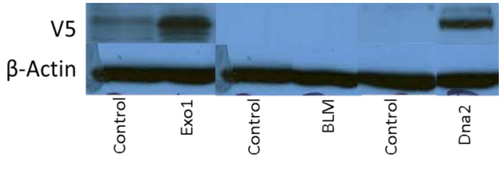

Figure 2. Cell lysates from transfected 293t cells lines were analyzed by Western Blot. EXO1 and DNA2 overexpression was achieved. BLM overexpression plasmid is not functioning properly.

Conclusions

The BLM plasmid needs to be evaluated to understand why transfected cells are not overexpressing the protein. Once we have three working plasmids we will obtain three stable cell lines that overexpress a different protein; BLM, DNA2, EXO1. The next step of this project will be doing a microarray or a transcriptome analysis of each of the cell lines to identify the key node in the expression network of the three proteins.

Understanding the homologous recombination pathway in its entirety, specifically the end resection part which is its rate limiting step, is crucial to identify gene and protein targets that can be altered by the use of drugs. This will hopefully allow us to abolish this pathway and the drug resistance it confers to malignancies. After the transcriptome analysis is done future studies may include using drugs that target the end resection pathway and different DNA damage agents that are used as chemotherapeutics to see if they enhance the efficiency and abolish drug resistance.

Hopefully afterwards new drugs may be tested in clinical trials and will increase the efficiency of existing treatments thereby lowering the required dose and by consequence the side effects related to that treatment.

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