Exploring a Link between Histone Demethylases and Alternative Polyadenylation

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ABSTRACT

Many recent reports in both mainstream and scientific media have centered on the concept that "DNA is not destiny". The key component of this phenomenon is epigenetics. Gene expression is regulated epigenetically on many levels. One key mechanism is the addition and removal of modifications to the N-terminal tails of histones. Jhd2 is a lysine demethylase found in *Saccharomyces cerevisiae*. It is the only demethylase capable of removing the activating H3K4me3 mark. Many epigenetic regulators have been shown to work as part of one or more complexes. Although Jhd2 and its human homolog, PLU1, are both major players in transcription in yeast and humans, they have not been comprehensively reported as being associated with any established complexes. In an effort to study the involvement of histone demethylases in cellular processes, we have affinity purified a TAP-tagged version of Jhd2. We found that it purifies with multiple components of yeast polyadenylation machinery. Specifically, Jhd2 co-purifies with Cpf11 and Yra1 but not Clp1. Recent reports note that Yra1 competes with Clp1 for binding Cpf11 resulting in alternative polyadenylation over canonical polyadenylation. The link between polyadenylation and demethylases has been recorded in plants but not in yeast or mammals.

PLU1, a human homolog of Jhd2, is upregulated in cancer tissue but not in normal tissue. This makes it an ideal target for cancer therapies. In addition to our yeast polyadenylation studies, we have found that PLU1 expression has an effect on the alternative polyadenylation (APA) of the oncogene CCND1. This alternative polyadenylation results in a loss of miRNA sites and therefore allows CCND1 to escape degradation by the RISC complex. Studies have shown that this form of APA is used commonly in cancer tissues as a mechanism for oncogenes to elude the normal degradation machinery in the cell. This phenomenon results in increased cell proliferation and tumor progression. We have developed a system for identifying transcripts whose APA is directly affected by PLU1 expression. Our system involves a comparison of gene expression and protein expression profiles from normal and PLU1 knockdown cell lines. Thus far, we have identified RAD23B as a potential target.

By elucidating the novel mechanism of histone demethylase involvement in APA in yeast and human cells, we will attain a better understanding of the mechanisms by which cancer progresses. These studies have the potential to lead to development of new drugs to treat cancer or novel uses for existing drugs targeted at the processes outlined here.

INTRODUCTION

The field of epigenetics aims to classify and explain the heritable changes affecting gene expression that do not involve primary DNA sequence. These include DNA methylation, histone modification and microRNAs (Figure 1)[1]. Key amongst histone modification is the H3K4me3 mark. This mark is associated with active gene transcription. Only one family of enzymes has been shown to remove this activating mark: the JARID1 family of histone demethylases. PLU1 is a member of this family that has been shown to be expressed in breast cancer cell lines, but not in normal adult tissues.[2] Knockdown studies showing that PLU1 deficient cells show significantly less tumor formation in a syngeneic mouse cancer model have indicated that PLU1 is an excellent target for anti-cancer therapies.[3] They exact mechanism of PLU1's involvement in cancer, however, is not known. Our studies have shown that PLU1 is potentially involved in alternative polyadenylation (APA) of oncogenes. By helping oncogenes select for shorter transcripts, PLU1 could be contributing to the loss of regulator miRNA sites. The ultimate result of this APA is overexpression of oncoprotiens. JHD2 is Only JARID1 homologue in yeast. We are combining studies in human cell lines and in yeast in order to elucidate the mechanism of PLU1's involvement in APA.

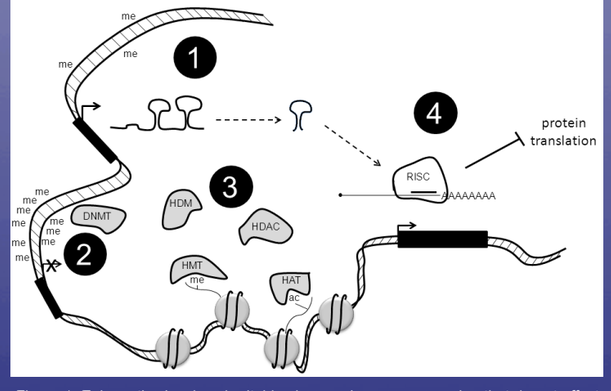

Figure 1. Epigenetics involves heritable changes in gene expression that do not affect the primary DNA sequence. The most well-documented incidences of epigenetics occur through DNA methylation, histone modification and microRNA expression.

RESULTS

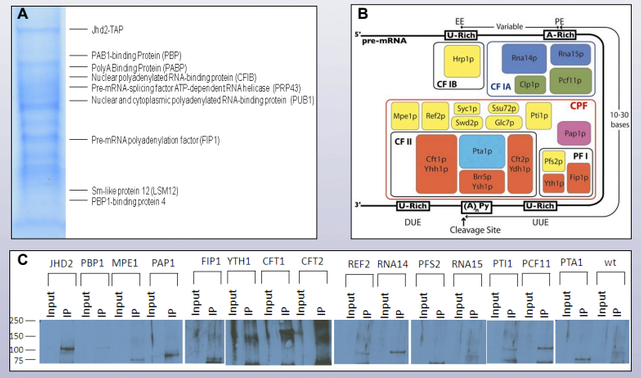

Figure 2. (**A**) Affinity purification of JHD2-TAP followed by mass spectrometry identifies many polyadenylation machinery component. (**B**) Yeast polyadenylation machinery. (**C**) Co-IP verification of polyA component interaction with JHD2.

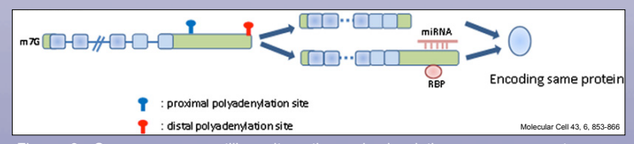

Figure 3. Oncogenes can utilize alternative polyadenylation as a means to escape regulatory miRNAs resulting oncoprotein overexpression.

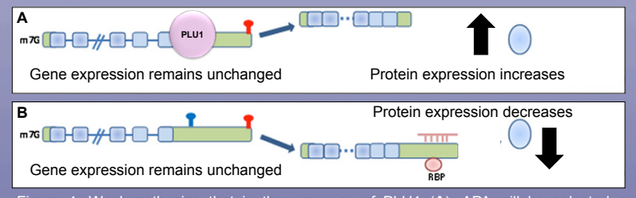

Figure 4. We hypothesize that in the presence of PLU1 (**A**), APA will be selected on oncogenes which will result in less miRNA regulatory sites and more protein. In PLU1 knockdown cells (**B**), we expect to see longer oncogene transcripts with miRNA regulatory sites and lower protein expression.

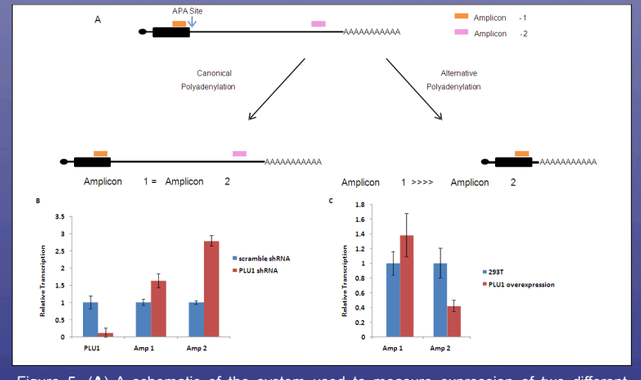

Figure 5. (**A**) A schematic of the system used to measure expression of two different polyadenylated forms of CCND1. (B) Knockdown of PLU1 results in an increase in the longer form of CCND1. (C) Overexpression of PLU1 results in a decrease in the shorter form of CCND1.

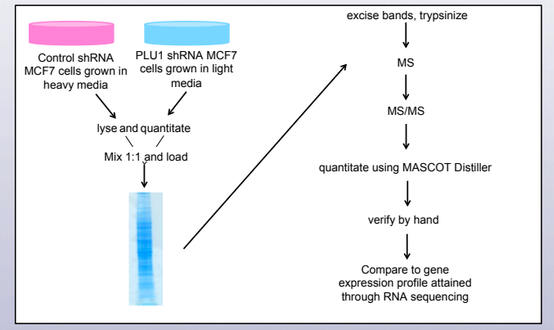

Figure 6. Workflow for identifying candidates of PLU1-mediated APA. In order to study protein expression in normal and PLU1 knockdown MCF7 cells, we used the Stable Isotope Labeling of Amino acids in Culture (SILAC) technique shown here. We then compared SILAC results to RNAseq results to identify changes in protein levels but not gene expression levels.

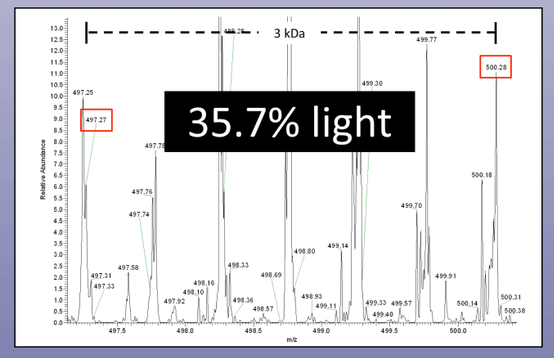

Figure 7. SILAC mass spectrometry results show that Rad23B protein expression is downregulated in PLU1 knockdown cells compared to normal MCF7 cells. Independent verification of the area under the 497.27 kDa peak (knockdown cells) compared to the 500.28 kDa peak (control cells) reveals that the absence of PLU1 results in a two fold decrease in protein expression.

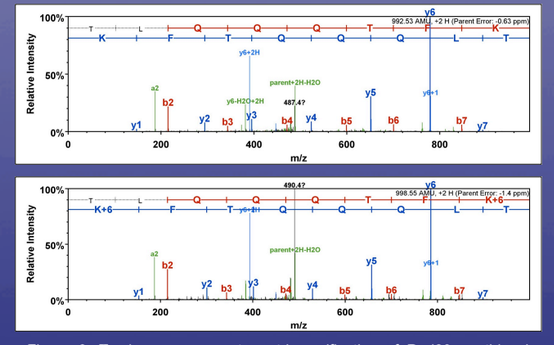

Figure 8. Tandem mass spectrometric verification of Rad23 peptides in Figure 7.

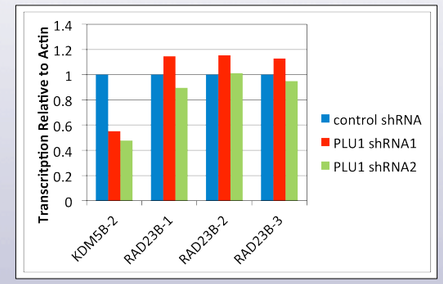

Figure 9. Realtime PCR verification of PLU1 knockdown and RAD23B expression. Three different rtPCR primers targeting the coding region of RAD23B were used to measure its expression level in control versus PLU1 knockdown cell lines. RAD23B levels did not change significantly as was seen in our RNAseq data.

CONCLUSIONS

- Yeast
  - Jhd2 binds polyA machinery.
  - Jhd2 specifically binds the CFIA component which is associated with selection of alternative polyA sites.
- Human
  - PLU1 interacts with peripheral components of the polyA machinery.
  - PLU1 contributes to APA of CCND1 in breast cancer cell lines.
    - PLU1 appears to bind more readily at APA sites rather than canonical polyadenylation sites.
  - Using a mixture of protein expression and gene expression techniques allows identification of APA targets of PLU1

FUTURE DIRECTIONS

- Yeast
  - Epistasis analysis of JHD2 and APA machinery
  - APA assay
  - RNA binding
  - ChIP
- Human
  - RNA-ChIP
  - RIP
  - Northern blotting verification of APA candidates
  - Western blotting verification of APA candidates
  - ChIP
  - Extended candidate discovery

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