Estrogen receptor interaction with Mediator of DNA Checkpoint 1 (MDC1) mediates epigenomic remodelingand gene regulation in ILC cells

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Abstract

Mediator of DNA damage checkpoint 1 (MDC1) is a protein which regulates how cells repair DNA damage. However, in invasive lobular carcinoma (ILC), MDC1 is "hijacked" by the estrogen receptor (ER), creating new ILC-specific functions for MDC1 in controlling how genes are turned on or off. ER-MDC1 together may be important for how ILC cells respond to the hormone estrogen, and resist anti-estrogen treatments. To better understand the ILC-specific activity of ER-MDC1, we performed experiments to identify other cellular proteins that partner with ER-MDC1, and compared MDC1 partners in ILC cells versus other breast cancer cells. We found that in ILC cells, MDC1 partners are shifted away from DNA repair proteins (as in other cancer cells) and are instead utilized to control gene expression. This work can help define anti-estrogen response and resistance in ILC cells, and lead to new therapies targeting the specific functions of ER-MDC1 in ILC cells.

Scientific Abstract

Estrogen receptor α (ER) has unique regulatory activities in invasive lobular carcinoma (ILC) cells associated with endocrine response and anti-estrogen resistance, which we linked to ILC-specific interaction between ER and Mediator of DNA Damage Checkpoint 1 (MDC1). MDC1 is a cornerstone of DNA damage repair yet in ILC cells has a novel, critical role in ER-mediated gene regulation. Defining this putative ILC-specific ER co-regulator function of MDC1 may identify the mechanisms underpinning ILC-specific functions of ER. We profiled the ER and MDC1 cistrome in ILC cells and found that at ER-regulated genes, ER binds distal enhancers, while MDC1 binds promoters, suggesting that MDC1 may facilitate the action of ER-bound enhancers at target gene promoters. To understand how MDC1 may regulate enhancer/promoter regulation, we performed MDC1 immuno-precipitation mass spectrometry (IP-MS) in ILC vs invasive ductal carcinoma (IDC) cell lines to profile partners that mediate ER-MDC1 activity. In total, we identified 2,221 MDC1-interacting proteins. IDC cell lines present preferential MDC1 association with DNA repair proteins. In ILC cell lines, MDC1 additionally associated with a cohort of epigenomic regulators. We identified 234 epigenomic partners associated with MDC1 with 66 of these proteins also interacting with ER. Importantly, treatment of MM134 with ER-antagonist fulvestrant remodeled MDC1 associated proteins to mirror that seen in IDC cells. To identify MDC1 critical epigenomic partners, we are undertaking an siRNA screen of 228 putative partners from IP-MS for their role in ER-driven phenotypes. We further explored how MDC1 regulates the epigenome and profiled chromatin accessibility by MNase-seq after MDC1 or ER knockdown, which suggests that MDC1 plays distinct roles in chromatin remodeling in ILC cells. Taken together, ER interaction with MDC1 may build gene regulatory complexes at ILC-specific ER target genes that facilitates chromatin remodeling, gene regulatory, and ultimately endocrine response and resistance in ILC.

Figure 1:

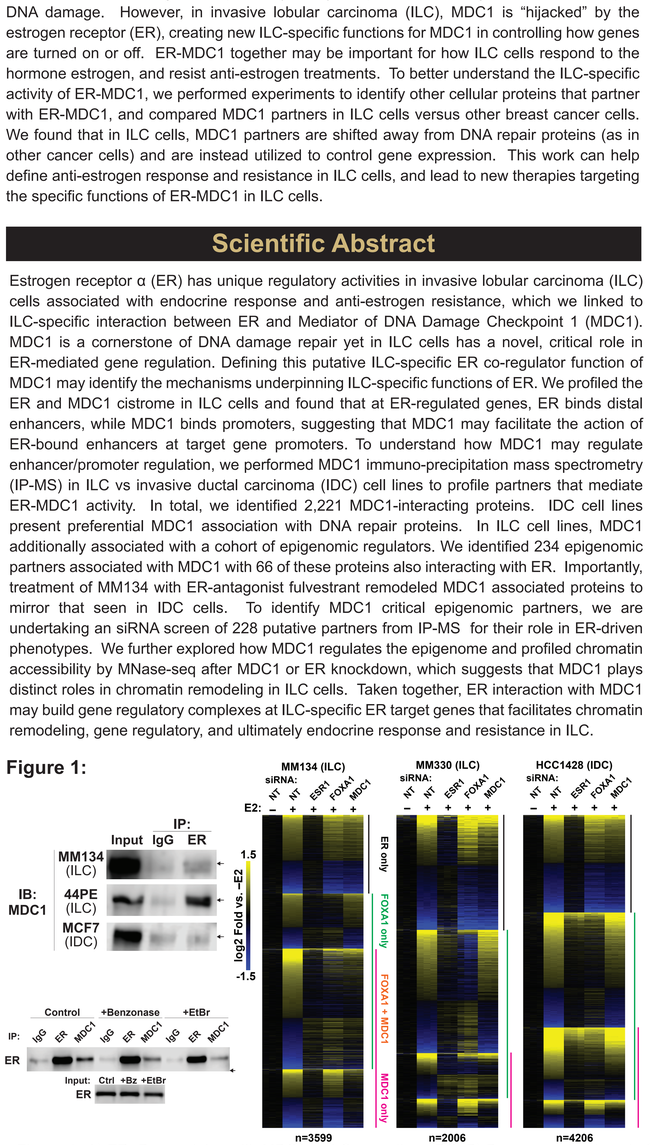

Figure 1: A) ER-IP associates with MDC1 in ILC more than IDC. B) Benzonase treatment of ER and MDC1 IP samples show the interaction is not due to DNA scaffolding. C) ER target genes in each cell line were identified by E2 regulation. Additionally, MDC1 and FOXA1 targets defined by E2 were reversed with siRNA treatment.

Co-operative model of ER and MDC1 cistromic regulation

Figure 2:

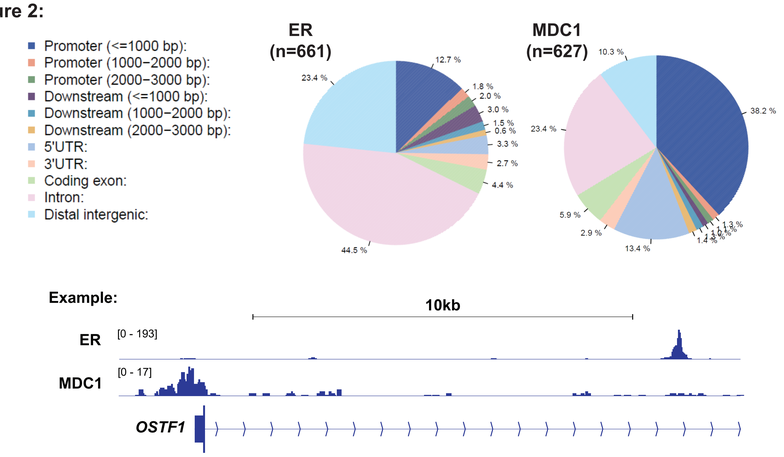

Figure 2: ER preferentially associates with distal enhancer elements whereas MDC1 associates with promoter elements determined by Cut&Run. An example of these associations is shown for osteoclast stimulating factor 1 (OSTF1)

MDC1 protein interactions as determined by IP/MS

Figure 3:

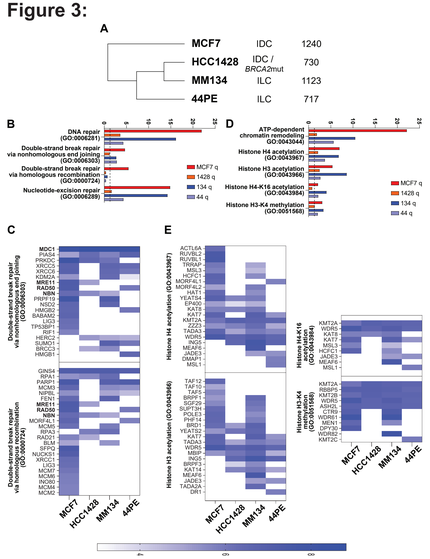

Figure 3: A) Hierarchical clustering and total number of MDC1 bound proteins identified. B-C) Enrichment of DNA damage response (DDR) pathways was present in all cell lines, but more so in IDC cell lines. Notably, the MRN complex is not constituitevely assocaited with MDC1 in ILC. D-E) ILC lines showed MDC1 increased associations with chromatin remodeling, particularly histone acetylation, compared to IDC cell lines.

Figure 4:

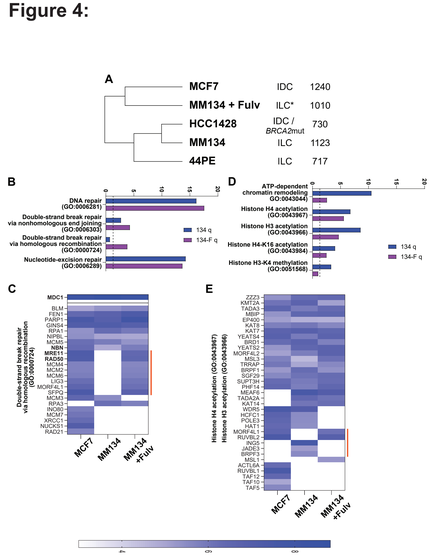

Figure 4: A) Hierarchical clustering of fulvestrant treated MM134 cells (MM134-F) are more similar to MCF7 than parental. B-C) MM134-F regain associations with MRN and other DDR proteins. D-E) MM134-F lose partners associated weith chromatin remodeling. Together, these suggest that ER degradation allows MDC1 to fulfill a more canonical role in the MM134 cell line.

Chromosomal availability it altered by ER and MDC1

Figure 5:

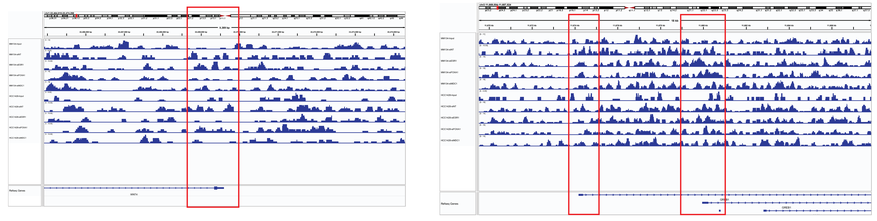

Figure 5: MNase-seq analysis of MM134 and HCC1428 cells shows differential access to chromatin. The 150mM salt fraction is depicted and shows variable enrichment associated when ER, MDC1, or FOXA is depleted.

siRNA screening of epigenomic regulators

Figure 6:

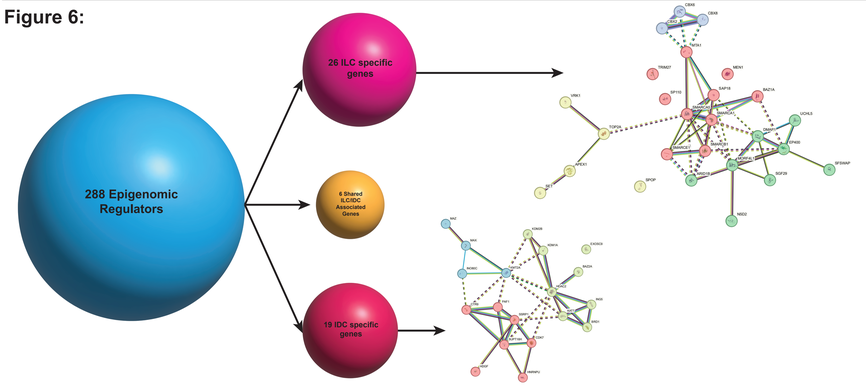

Figure 6: 288 epigenome regulators known to interact with MDC1 were screened by siRNA in MM134, 44PE, MCF7, and HCC1428 cells +/-E2. Cellular number after two doubling times was determined. Lethal estrogen responsive genes were identified. STRING analysis of genes with Kmeans clustering was performed.

Conclusions and Future Directions

Conclusions

-ER binds distal enhancer elements whereas MDC1 binds promoter elements.

-ER exploits MDC1 promoter function in ILC, leading to novel regulation of epigenomic mediators.

  -In ILC, MDC1's role as a transcriptional regulator is promoted whereas it's canonical role as a DDR scaffold is inhibited.

  -Degradation of ER with fulvesterant may return MDC1 to it's primary DDR function.

-ER and MDC1 work co-operatively to regulate chromosomal availability of transcription factors, likey through histone methylation and acetylation.

-MDC1 co-epigenomic regulators can be targeted leading to estrogen sensitive growth inhibition.

Future Directions

-Define genetic loci co-operatively managed by ER and MDC1.

-Identify the sites of histone methylation and acetylation of ER responsive genes.

-Identify druggable epigenomic regulators that can inhibit ILC growth.

  -*In vitro* and *in vivo* testing of compounds for growth inhibition.

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References

Sottnik & Bordeaux. Molecular Cancer Research 2022.