Title: CARM1-mediated methylation of ZNF703 regulates its oncogenic functions in estrogen receptor positive breast cancer

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Scientific Abstract:

ER-positive (ER+) breast cancer, characterized by estrogen receptor expression on tumor cells, constitutes approximately 70% of all breast cancer cases. Identifying specific oncogenes and elucidating relevant oncogenic mechanisms are critical for developing effective therapies. *ZNF703*, a NET/N1Z family transcription factor, drives 8p12 locus amplification found in 10-20% of ER+ breast tumors. *ZNF703* has been reported as an oncogene that promotes ER+ breast tumorigenesis in vitro. However, its regulation and *in vivo* oncogenic function remain unclear. We showed that *ZNF703* expression is induced by estradiol in ER+ cell lines. ShRNA-mediated ZNF703 knockdown significantly reduced cell proliferation in MCF7 and BT474 cells. ZNF703 is post-translationally modified, which may affect its nuclear localization as well as its functions. Using mass spectrometry, we identified two asymmetrically di-methylated arginine residues (R544 and R580) on ZNF703 in human breast tumors. Protein arginine (R) methylation is catalyzed by protein arginine methyltransferases (PRMTs), which are classified into Type I, II, and III based on the type of methyl-R they produced. Among these, CARM1, a Type I PRMT, catalyzes asymmetric di-methylation of arginine residues. We showed that ZNF703 is methylated by CARM1 in *in vitro* methyltransferase assay. Furthermore, ZNF703 protein levels were reduced in CARM1 knockout MCF7 cells, accompanied by an increased degradation rate, suggesting that ZNF703 may be stabilized by CARM1. Given the lack of an ER+ breast cancer mouse model, we derived an ER+ MG1 rat cell line from estrogen-induced rat breast tumors. Consistent with our findings in human ER+ cell lines, ZNF703 was validated as an estrogen-induced gene in MG1 cells. ZNF703 knockout in MG1 cells impaired cell proliferation and colony formation. Functional rescue experiments demonstrated that re-expression of wild-type ZNF703 fully restored cell growth, whereas the methylation-deficient mutants ZNF703 R580K and R544K only partially rescued the proliferation defect. Our results implicate that CARM1-mediated ZNF703 methylation may regulate its stability and oncogenic functions. Given that patients diagnosed with 8p12 locus amplification caused by *ZNF703* typically have poor prognosis, our MG-1/ACI rat syngeneic model would enable the investigation of *ZNF703*'s oncogenic functions in vivo.

Written Lay Abstract:

The most common type of breast cancer is estrogen receptor-positive (ER+). To make better treatments for ER+ breast cancer, researchers want to know what genes (sections of DNA) allow this cancer to grow.

For this study, researchers looked at the gene *ZNF703*. Past studies on cells in the lab found that *ZNF703* can "turn on" and start pathways causing cancer to grow, but researchers are not sure if this works the same way in the body.

The researchers in this study used cells and rats to answer this question. They found that *ZNF703* is turned on by estrogen in ER+ breast cancer cells. When researchers added a genetic tool (called shRNA) to the cells, the amount of ZNF703 (the protein that *ZNF703* gives instructions to make) was lowered, and cancer cells grew slower.

The researchers then studied how ZNF703 protein works in breast cancer cells. They found that ZNF703 was changed by a process called "arginine methylation", which changed where the protein goes and acts in the cell. They also found that ZNF703 was stabilized, meaning it is not broken down in the cell. These changes to ZNF703 were made by another protein called CARM1.

When the researchers removed CARM1 from cells, the amount of ZNF703 protein lowered and the current ZNF703 proteins broke down faster. This tells us that ZNF703 needs CARM1 to stay stable and cause cell growth.

Researchers then studied ZNF703 in rats with ER+ breast tumors and found that estrogen turns on *ZNF703* in living animals. Removing ZNF703 protein in the rats lowered cancer cell growth, just as was seen in cells in the lab. Adding ZNF703 that could not be changed by CARM1 increased cancer cell growth again, but not by much.

This study tells us that in ER+ breast cancer, estrogen turns on *ZNF703*, which leads to more ZNF703 protein, which leads to more cancer cell growth. ZNF703 protein needs CARM1 to stay stable and lead to cancer growth. Future ER+ breast cancer treatments could target the protein CARM1 and the gene *ZNF703* to stop this pathway and stop cancer cell growth.

Visual Lay Abstract:

How breast cancer grows: ZNF703 and CARM1

ER+ is the most common type of breast cancer

The estrogen receptor (ER) is a protein that attaches to the hormone estrogen and tells cancer cells to grow. ER+ breast cancer cells have more ER. In this study, researchers looked at how other proteins affect ER and cause breast cancer to grow.

Estrogen increases ZNF703 protien

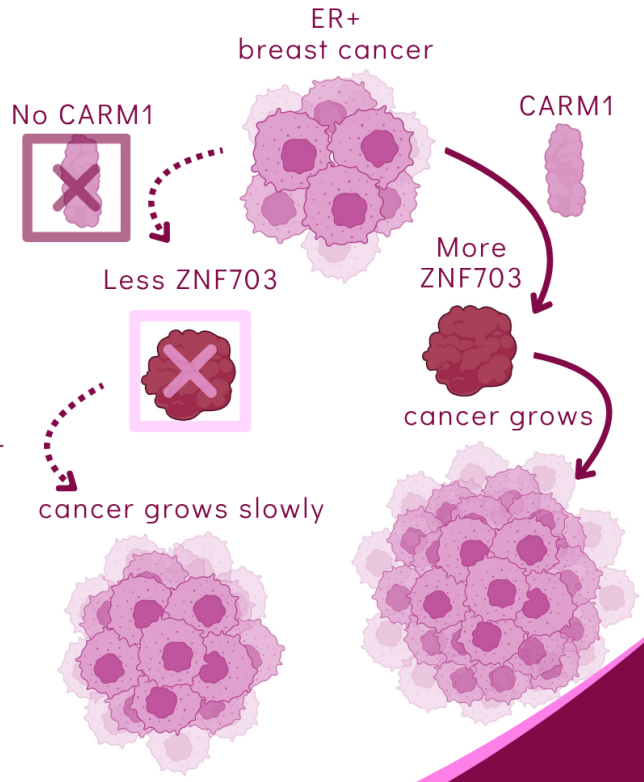
Researchers found that estrogen, a hormone, increases the amount of ZNF703 protein in cancer cells

Lowering ZNF703 slows cancer growth

When researchers lowered the amount of ZNF703 protein, cancer cells grew slower

Taking away CARM1 lowers ZNF703 and could be used to treat cancer

When researchers took away a protein called CARM1, ZNF703 broke down and cancer grew slower



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