ICR The Institute of Cancer Research

E-cadherin loss imparts mitotic vulnerabilities rendering breast cancer cells synthetic lethal to crizotinib and up-regulation of Src signalling reverses this effect

William W. Yang, Rachel Brough, Feifei Song, Stephen Pettitt, Andrew N. J. Tutt, Christopher J. Lord

Lay Abstract

The E-cadherin molecule is often missing in lobular breast cancer cells. While its main job is to help cells stick together, it also plays a role in cell division. Previous work in our lab have found that a drug called crizotinib can be effective against cancers that do not have E-cadherin. Our current work has discovered that crizotinib causes problems in cell division, resulting in mistakes that can ultimately cause the cell to die. We identified a molecule called *CSK* that regulates this process and when *CSK* is turned off, the cancer cells become resistant to crizotinib. We also find that *CSK* may be involved in a pathway that controls the formation of important structures during cell division. This research could lead to new treatments that targets these vulnerabilities in lobular breast cancer.

Introduction

- Loss of E-cadherin (*CDH1*) is the pathognomonic alteration in lobular breast cancer.
- We have previously demonstrated synthetic lethality between the clinical *ROS1* tyrosine kinase inhibitor crizotinib and E-cadherin loss.
- Genetic perturbation screens of E-cadherin defective breast cancer cells exposed to crizotinib identified increased dependencies on genes controlling mitotic processes.
- E-cadherin has roles beyond cell adhesion and is known to regulate mitotic spindle orientation as well as centrosome clustering.
- Our top validated crizotinib resistance gene hit was *CSK* - the negative regulator of Src-family kinase (SFK) signalling.
- In this poster we describe the crizotinib synthetic lethal mechanism, linking E-cadherin loss with dysregulation of Aurora-A kinase localisation and provide a possible resistance pathway mediated through loss of *CSK*.

1. Uncovering novel mediators of crizotinib resistance

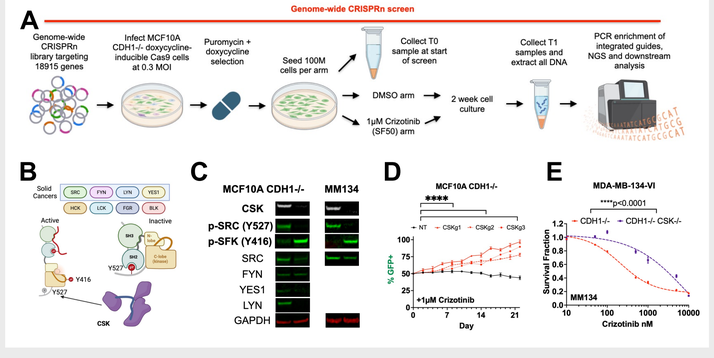

Figure 1. Genome-wide CRISPR screen uncovers novel mediators of crizotinib resistance in E-cadherin deficient cells.
A. Schematic of CRISPRn screen. **B.** Knockout of *CSK*, negative regulator of Src family kinases (SFK) validated as a top resistance hit. **C.** *CSK-/-* lines were generated and effects on SFK activity defined by western blot. **D.** Knockout of *CSK* using three different CRISPR guides validated crizotinib resistance in competition assays. **E.** ILC MM134 *CSK-/-* cell line was crizotinib resistant.

2. E-cadherin loss weakens centrosome functions

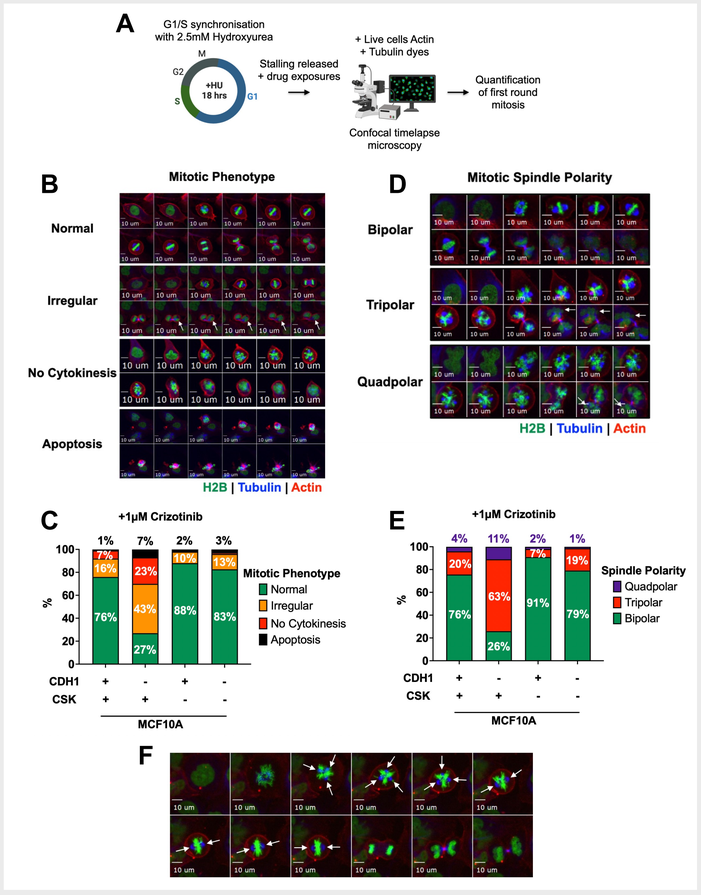

Figure 2. E-cadherin loss exacerbates formation of multipolar mitotic spindles after crizotinib exposure and up-regulation of SFK-activity rescues this defect.
A. Cells were synchronised with Hydroxyurea and confocal time-lapse microscopy carried out. **B+C.** Cells exposed to crizotinib were classified into four phenotypes. **D+E.** Quantification of spindle polarity prior to anaphase. **F.** *CSK-/-* cells were able to cluster fragmented spindle poles, restoring bipolarity.

3. E-cadherin and SFKs modulates centrosomal Aurora-A

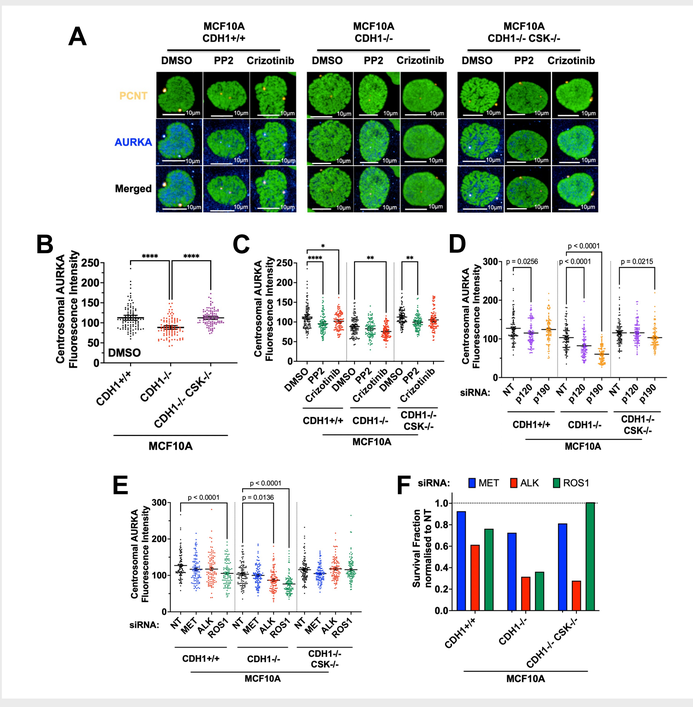

Figure 3. E-cadherin and SFK-activity modulates centrosome maturation by promoting Aurora-A kinase localisation during late G2/early M phase.
A. Cells expressing H2B-GFP were stained for Pericentrin (PCNT) and Aurora-A kinase (AURKA). **B.** Fluorescence intensity of AURKA at the centrosome was quantified. **C.** Centrosomal AURKA quantification following exposure to PP2 (pan SFK-inhibitor) or crizotinib. **D.** p120 or p190 RNAi reduced centrosomal AURKA in CDH1-/- cells. **E.** ROS1 or ALK RNAi reduced centrosomal AURKA in CDH1-/- cells. **F.** ROS1 RNAi synthetic lethality was reversed following CSK-loss.

Conclusions

- Loss of E-cadherin weakens centrosome maturation through reducing Aurora-A kinase localisation during late G2/early M phases of the cell cycle.
- This defect following E-cadherin loss can be targeted through ROS1 and ALK inhibition with crizotinib.
- Up-regulation of Src-family kinase activity confers resistance through restoration of Aurora-A kinase expression.

Contact

Will Yang, PhD
Gene Function Lab
Breast Cancer Now Toby Robins Research Centre
The Institute of Cancer Research
237 Fulham Road London SW3 6JB
will.yang@icr.ac.uk

Acknowledgments

This work was funded by Programme Grants from Breast Cancer Now as part of Programme Funding to the Breast Cancer Now Toby Robins Research Centre.

BREAST CANCER NOW The research & support charity