CDC25A overexpression confers sensitivity to WEE1 kinase inhibition

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Background

CDK Mediated Cell Cycle Regulation:

- Cyclin-dependent kinases (CDKs) regulate the cell cycle

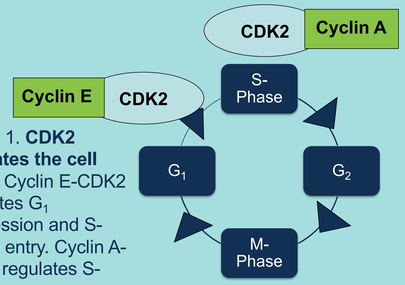


Figure 1. **CDK2 regulates the cell cycle.** Cyclin E-CDK2 regulates G_1 progression and S-Phase entry. Cyclin A-CDK2 regulates S-Phase.

- CDK2 is regulated by inhibitory phosphorylation via WEE1 and dephosphorylation via CDC25A

Figure 2: **WEE1 and CDC25A regulate CDK2 activity.** WEE1 phosphorylates tyrosine 15 (Y15) and threonine 14 (T14), inhibiting CDK2, while CDC25A removes these phosphates, activating CDK2

Therapeutic Potential of Over-expressed CDC25A:

- Preliminary results suggest that overexpression of CDC25A, which is common in many cancers, sensitizes cells to treatment by WEE1 inhibition

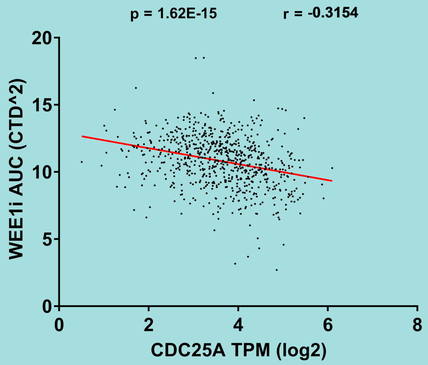
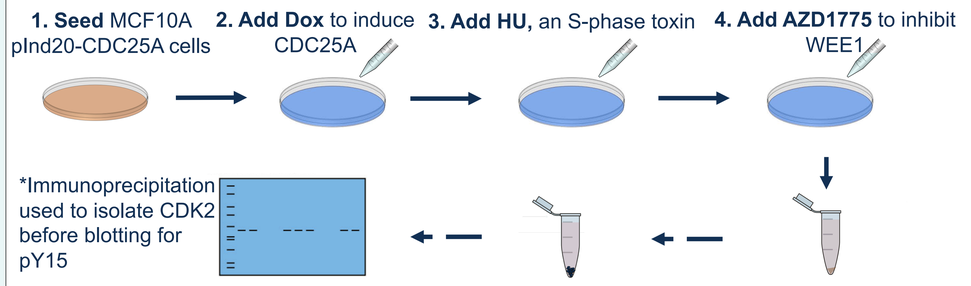


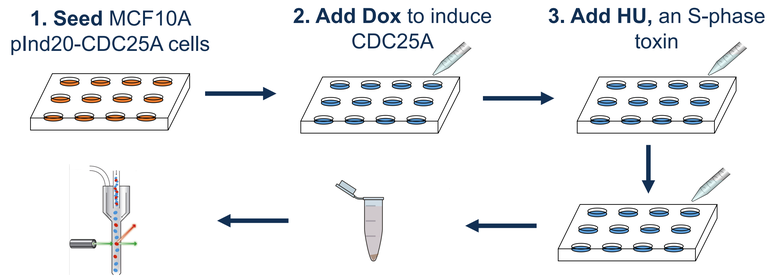
Figure 3: **CDC25A sensitizes many cancer types to WEE1i.** As part of the DepMap project, cell sensitivity to AZD1775 was tested against different levels of CDC25A transcript expression in 739 different cancer types.

Methods

Protein Levels via Western Blot



Protein Levels via Flow Cytometry



Results

Endogenous CDC25A enhances reduction in Y15 phosphorylation via WEE1i

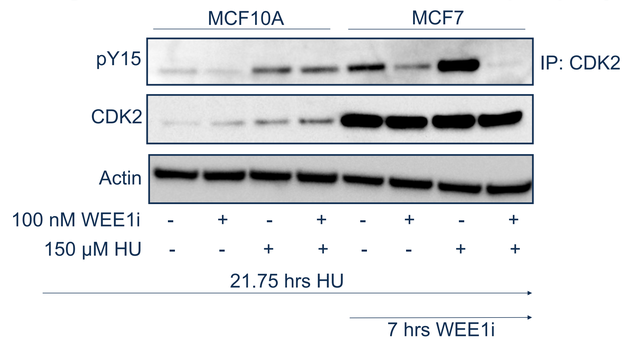


Figure 4: **pY15 decreases more in MCF7 cells** (high CDC25A) as compared to MCF10A cells (low CDC25A)

Dox induces CDC25A in MCF10A pInd20-CDC25A cells

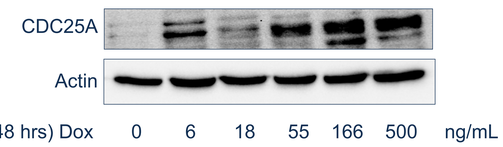


Figure 5: **Dose response** of MCF10A pInd20-CDC25A cells to Doxycycline over 48 hours.

Induction of CDC25A reduces Y15 phosphorylation on CDK2

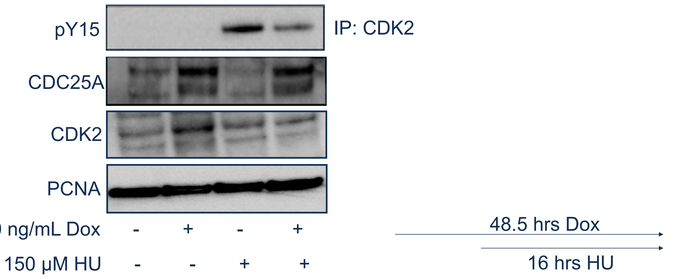


Figure 6: **pY15 decreases after CDC25A is induced** via treatment with dox

Lack of CDC25A induction causes no change in DNA damage

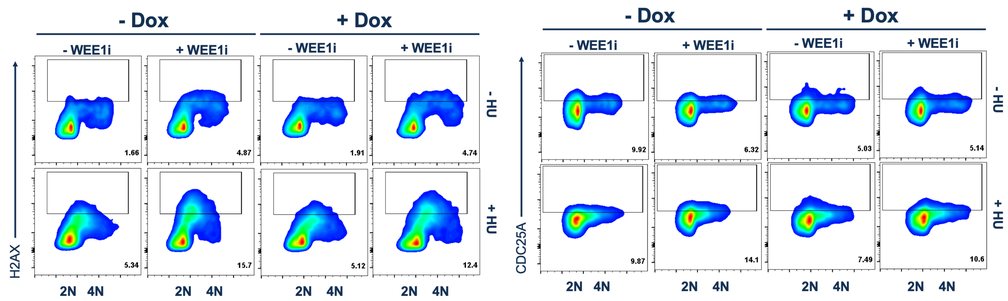


Figure 7: **No change in DNA damage** in cells with Dox vs without Dox. This is likely due to the lack of CDC25A induction. Cells were treated with Dox for a total of 46.5 hours, HU for a total of 22.5 hours, and AZD1775 for a total of 7 hours.

Objective

CDK activity has been shown to be abnormally high in many cancers, making CDKs great therapeutic targets. CDK2 has specifically been identified for its therapeutic potential due to the fact that inhibition of WEE1, a kinase that phosphorylates and inhibits CDK2, leads to CDK2-driven replication stress, and eventually cell death. Our preliminary work suggests that elevated CDC25A levels correlated with sensitivity to the WEE1 inhibitor AZD1775 across many cancer types. Since CDC25A activity is elevated in some cancers, it's been hypothesized that elevated CDC25A could be a biomarker for tumors sensitive to treatment by WEE1 inhibition. We used a dox-inducible system in cells with low endogenous CDC25A, MCF10A cells, to determine if overexpression of CDC25A confers sensitivity to WEE1 inhibition.

Conclusions

- The reduction in inhibitory phosphorylation on CDK2 after treatment with WEE1i occurs at a higher degree in MCF7 cells, which have high endogenous CDC25A as opposed to MCF10A cells which have low endogenous CDC25A.
- CDC25A can successfully be induced in MCF10A pInd20-CDC25A cells when treated with Dox. While the dose response shows that induction starts at 6 ng/mL after 48 hours, success was had with concentrations of 200 ng/mL and higher over a period of 48 hrs.
- **Over-expression of CDC25A reduces CDK2 inhibitory phosphorylation** in cells treated with the S-phase toxin, HU.
- **Levels of DNA damage don't appear to change in cells treated with Dox vs not treated with Dox but CDC25A also appears to not be induced.**

Future Directions

- Troubleshoot CDC25A induction
- Determine if over-expressing CDC25A actually increases CDK2 activity
- Determine if CDC25A over-expression is a prognostic marker for sensitivity to WEE1i + HU in patient-derived xenograft cancer models

Acknowledgements

This work is funded by NIH/NCI 5 R01 CA193808-04. The Summer Undergraduate Research Program is supported in parts by UCIHP Jeff Metcalf Summer Cancer Research Fellowship Program, the Cancer Center Support Grant (CCSG) CURE Supplement: NCI 3P30CA015704, the Fred Hutch Internship Program, and individual labs/research groups.