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
- Cyclin E-CDK2 regulates G1 to S-Phase entry
- Cyclin A-CDK2 regulates S-Phase

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

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

Methods
Protein Levels via Western Blot
1. Seed MCF10A pln20-CDC25A cells
2. Add Dox to induce CDC25A
3. Add HU, an S-phase toxin
4. Add AZD1775 to inhibit WEE1

Protein Levels via Flow Cytometry
1. Seed MCF10A pln20-CDC25A cells
2. Add Dox to induce CDC25A
3. Add HU, an S-phase toxin

Results
Endogenous CDC25A enhances reduction in Y15 phosphorylation via WEE1i

Induction of CDC25A reduces Y15 phosphorylation on CDK2

CDC25A overexpression confers sensitivity to WEE1 kinase inhibition

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 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 pln20-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 overexpression is a prognostic marker for sensitivity to WEE1i + HU in patient-derived xenograft cancer models

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