Stem Cell Markers May Be A Predictive Biomarker for MLN0128 in Endometrial Cancer

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Abstract

The mTOR (mechanistic target of rapamycin) pathway is a critical mechanism for cell survival, making it a significant target for cancer treatment. Endometrial cancer cell lines exhibit variable sensitivity to mTOR inhibitor MLN0128. We collected IC50 values for MLN0128 across established endometrial cancer cell lines and found correlations between gene expression data and sensitivity. By investigating a network of genes correlated with sensitivity, potentially important genes and upstream regulators have been identified, including chemokines and cancer stem cell markers such as EPCAM, IL8, TNF, and VEGF, as well as SYK, a targetable tyrosine kinase that may contradict a key mechanism of MLN0128.

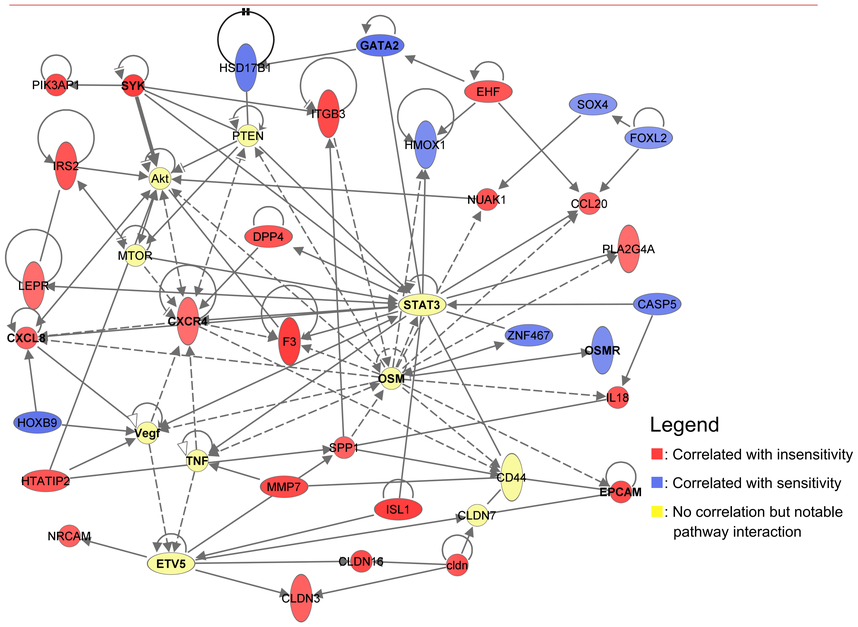
Background

MLN0128 is a second generation mTOR inhibitor that is currently in phase I clinical trial for combination therapy with bevacizumab in patients with advanced solid tumors. mTOR is a key nutrient-sensing pathway critical for nearly all cancers, particularly in endometrial cancers where PTEN (phosphatase and tensin homolog), an upstream regulator of mTOR, is frequently mutated. *In vitro* proliferation assays have demonstrated varied sensitivity to MLN0128 across established endometrial cancer cell lines. Determination of a biomarker predictive of sensitivity to MLN0128 may lead to more appropriate first-line therapy and new targets for combination therapy in endometrial cancer patients. We thus tested 6 endometrial cells lines which highlighted potential pathways that are responsible for MLN0128 sensitivity.

Methods

- MLN0128 IC50s determined via 96hr XTT cell proliferation assays
- mRNA gene expression data collected (via Broad-Novartis CCLE) - 19,000 genes
- Filter out genes with Spearman rank correlation coefficient $r < 0.7$, $r > -0.7$ - 2500 genes
- Filter out genes with fold change < 1.5 - 390 genes
- Construct network with Ingenuity Pathway Analysis (pictured) to identify important genes - 10 genes

Results



Gene of Interest	Correlated with:	Major Regulator(s), Interactions	Known Stem Cell Marker
SYK	Insensitivity	TNF, VEGF, IL8, Akt, STAT3,	No
OSMR	Sensitivity	VEGF, OSM, STAT3	No
IL8	Insensitivity	VEGF, Akt	Yes[1]
EPCAM	Insensitivity	OSM	Yes[2]
CXCR4	Insensitivity	TNF, IL8, VEGF, STAT3	Yes[1]
GATA2	Sensitivity	STAT3	Yes[3]
Additionally, TNF, VEGF, IL8, and STAT3 may also be potential stem cell markers.			

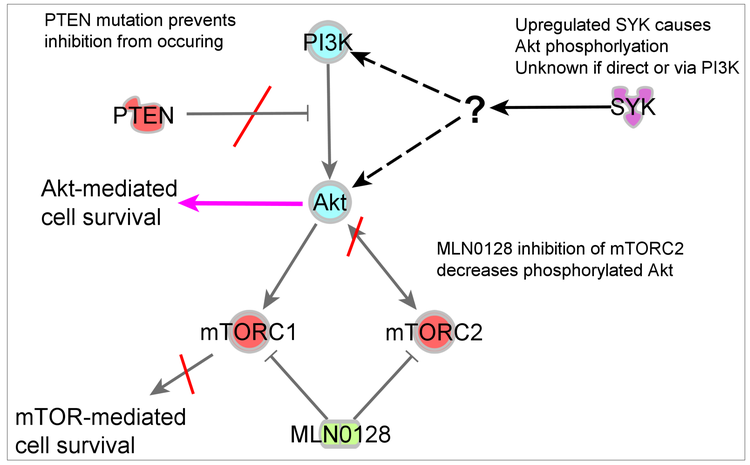
Endometrial cancer cell lines used and their IC50 values for treatment with MLN0128 are AN3CA, 1nM; RL952, 5nM; HEC1B, 7nM; ECC-1, 14nM; KLE, 17nM; and HEC1A, 28nM. AN3CA, RL952, and ECC-1 are PTEN mutants (per CCLE).

Major genes that are correlated with in/sensitivity or are upstream of multiple correlated genes include IL8, OSM, CXCR4, and VEGF, which have been previously associated with cancer stem cells. EPCAM is a known stem cell marker and GATA2 may be associated with differentiation.

SYK (spleen tyrosine kinase) is not strongly associated with cancer stem cells or cytokines already highlighted. However, increased levels of SYK have been associated with phosphorylation of Akt[4], which may be a cause for decreased MLN0128 efficacy, especially in PTEN-mutant cell lines. A simplified potential pathway for this interaction is pictured.

Conclusion

- Insensitivity to MLN0128 appears to be associated with cancer stem cell markers, as well as signaling proteins TNF, VEGF, IL8, OSM, and STAT3
- Sensitivity to MLN0128 may also be associated with SYK upregulation via Akt phosphorylation, especially in PTEN mutants
- Future research:
  - Determine if SYK inhibition affects MLN0128 sensitivity, and if so, determine a mechanism of action
  - Further investigational analysis of signaling proteins to see which are truly involved in MLN0128 sensitivity vs. coincidence



Above: PTEN mutations prevent inhibition of PI3K-Akt interaction, causing upregulation of Akt and mTOR prosurvival pathways. MLN0128 prevents mTOR-mediated survival mechanisms, but SYK may be causing Akt-mediated survival mechanisms to continue

References

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