Simultaneous knockdown of CD320 and LRP2 receptors is selectively toxic to cancer cells but not normal cells

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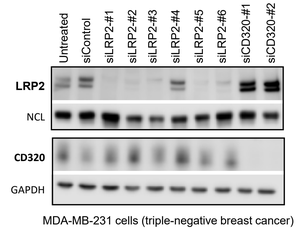
Abstract #1223

Introduction

- Porphyrins are known to concentrate in cancer and cancer-associated cells.
- Research was undertaken to understand the mechanism of action for the selective uptake of meso-tetra (4-carboxyphenyl) porphyrin (TCPP) in cancer cells (Elzi et al, FASEB J, 2021).
- We examined the structure of TCPP, structurally related molecules and their uptake mechanisms.
- We focused our efforts on two LDL-containing surface receptors, CD320 and LRP2, which import Vitamin B12 (cobalamin), which has a structure similar to a porphyrin.
- CD320 is the major cellular receptor for the uptake of cobalamin/Transcoballamin II.
- CD320 is overexpressed in cancer to meet increased demands for cobalamin.
- LRP2 also imports cobalamin/Transcobalamin II complexes into the cell, amongst more than 50 other ligands.
- LRP2 is highly expressed in the normal kidney tissue. Its role in cancer is poorly understood.
- Our group found that TCPP binds to and uses CD320 to enter cancer cells. We also found that, like CD320, LRP2 contributes to TCPP uptake in cancer cells.
- When we attempted to simultaneously knockdown expression of CD320 and LRP2 to examine their synergistic effects on TCPP uptake, we found that cancer cells died, while leaving normal cells unharmed.
- We set out to examine the effects of CD320 and LRP2 knockdown on a diverse set of cancer cell lines, to see if this could be a therapeutic approach for cancer.

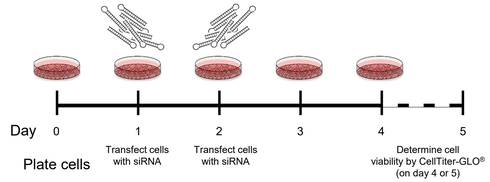
siRNAs effectively reduce CD320 and LRP2 levels

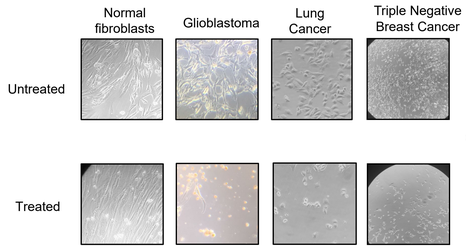


MDA-MB-231 cells (triple-negative breast cancer)

siRNAs targeting CD320 and LRP2 genes were designed and synthesized. siRNAs were transfected into cells using commercial transfection reagents. Protein knockdown levels were assessed by western bot.

siRNAs directed at CD320 and LRP2 are toxic to cancer cells

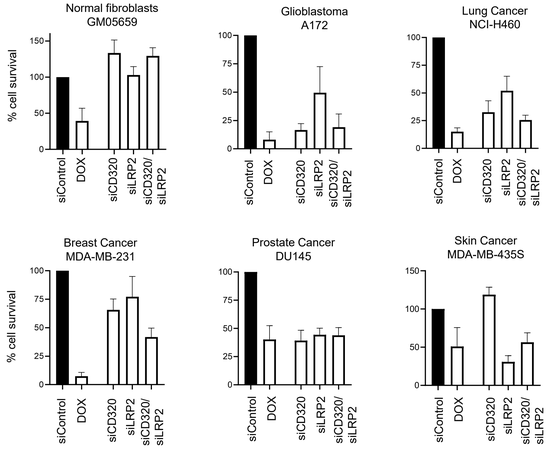




Cancer Cell types tested:
- Lung
- Brain
- Skin
- Prostate
- Breast

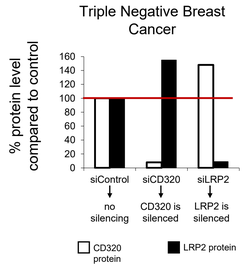
Cells are transfected with siRNAs. At the appropriate timepoint, cells are harvested for the Cell-Titer-GLO® assay, which measures ATP production as an indication of viability.

Diverse cancers are affected by CD320 and LRP2 knockdown



Notes: Cell growth of siControl was ~ 90-95% compared to cells that received no siRNA treatment.
Doxorubicin (DOX) served as a positive control.

Silencing both CD320 and LRP2 is essential

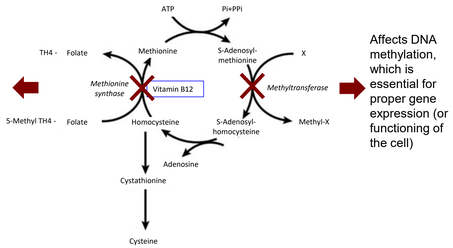


Silencing of only one receptor (CD320 or LRP2) in some cancer cells results in increased expression of the other receptor – a potential mechanism of resistance.

For efficient cancer cell killing both CD320 and LRP2 need to be silenced simultaneously so that cells are prohibited from potentially compensating and surviving.

Hypothesis for mechanism of cytotoxicity

Affects the generation of essential DNA building blocks, which is required for propagating new cells



Affects DNA methylation, which is essential for proper gene expression (or functioning of the cell)

We are currently testing the hypothesis that the simultaneous knock-down of CD320 and LRP2 leads to a cobalamin deficiency that is detrimental for cancer cells but can be better tolerated by normal cells.

Conclusions and future directions

- Silencing the receptors CD320 and LRP2 are toxic to a diverse range of cancer cell lines, while leaving normal cells unharmed.
- Silencing of one receptor can result in increased expression of the other, implicating a compensatory mechanism between the two receptors.
- We are testing the hypothesis that Vitamin B12 deficiency is a mechanism of action for the cytotoxic effects observed.
- We are expanding our testing of CD320 and LRP2 knockdown on cell survival in additional primary cells.
- We are implementing *in vivo* experiments to test the effect of CD320 and LRP2 knockdown on tumor growth.

Contact information

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