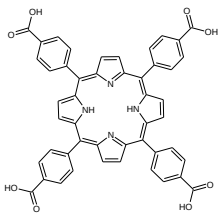
Meso-tetra (4-carboxyphenyl) porphyrin (TCPP) is taken up in cancer cells by the CD320 receptor

David J. Elzi[a], William E. Bauta[a], Jamila R. Sanchez[a], Trisha Das[a], Shweta Mogare[a], Peter Zannes Fatland[a], and Vivienne I. Rebel[a,b]

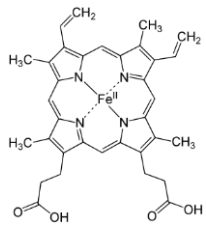
[a]bioAffinity Technologies, Inc., San Antonio, TX, USA
[b]Department of Cell Systems & Anatomy, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Introduction



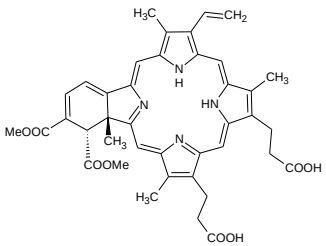
TCPP

- Porphyrins have long been known to preferentially accumulate in cancerous compared to normal tissue [1]
- bioAffinity uses the porphyrin TCPP in its lung cancer diagnostic CyPath® Lung [2]
- Research was undertaken to understand the mechanism of action for TCPP's selective uptake in cancer [3]
- Previous research in the field had suggested that the low density lipoprotein receptor (LDLR) was an avenue for porphyrin uptake in cancer cells [4]
- bioAffinity research showed that the LDLR was not a major pathway for TCPP uptake in cancer cells [3]
- In the search for alternative pathways for TCPP uptake, we compared the structure of TCPP with related molecules naturally occurring in the body and their mechanisms of uptake



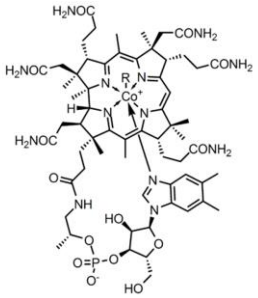
Heme

SLC46A1
SLC48A1
FLVCR1
FLVCR2



Benzoporphyrin analog

LDLR

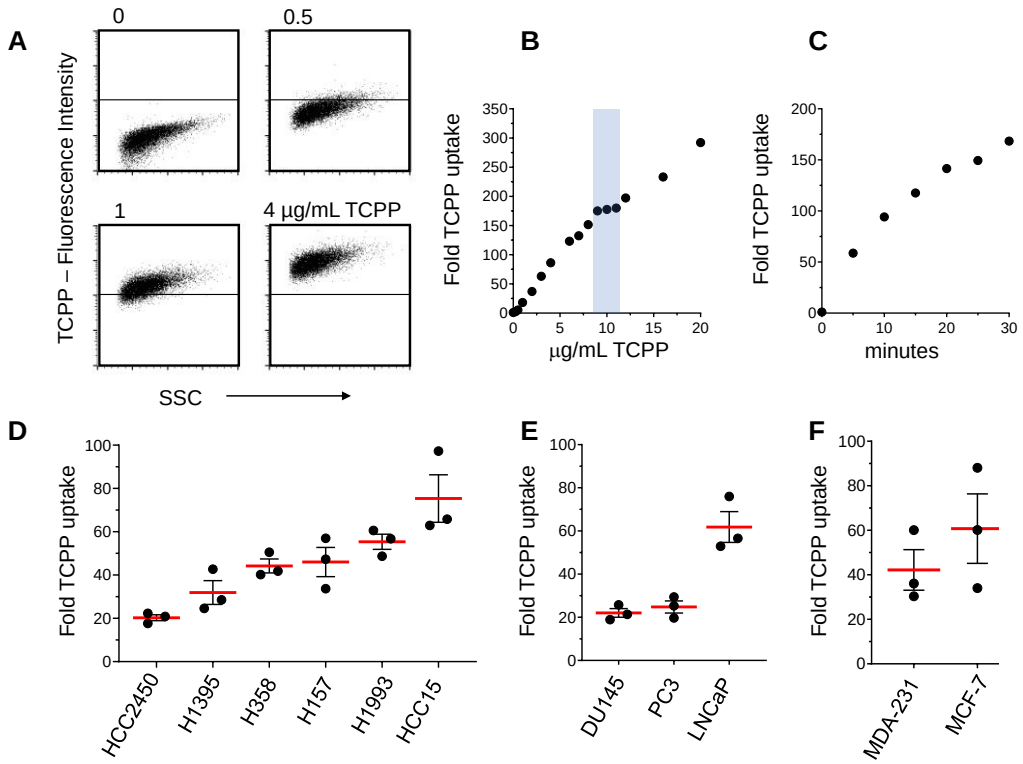


Cobalamin

CD320
LRP2
CUBN

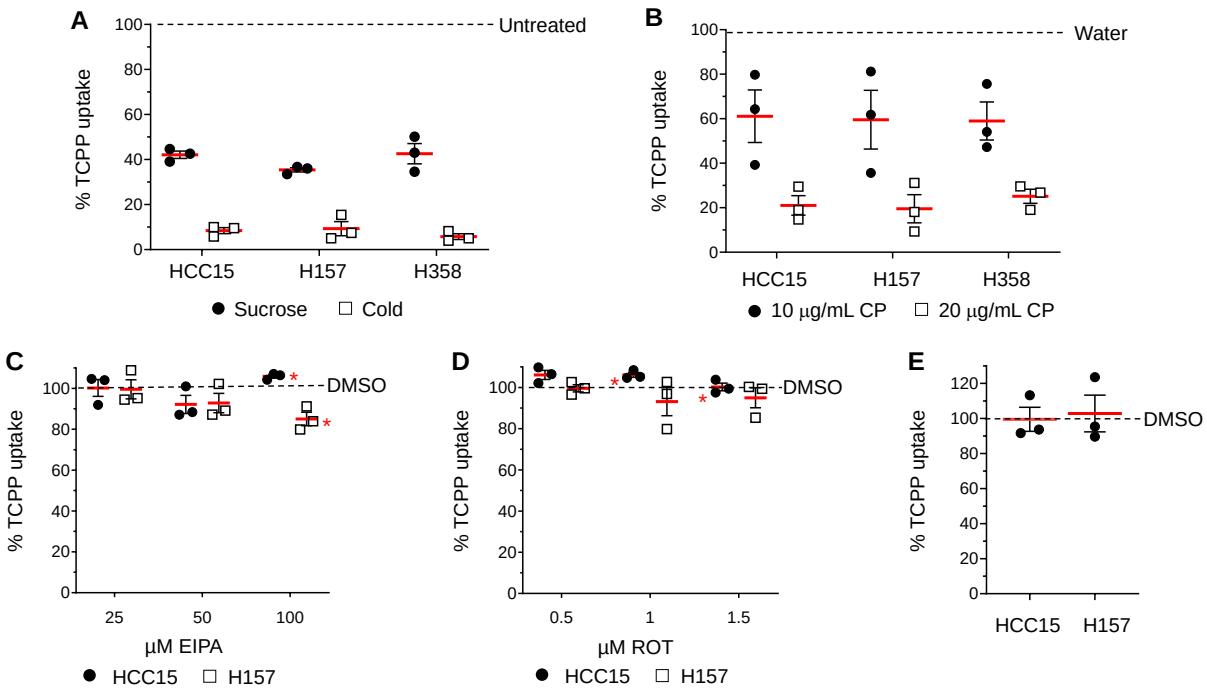
- We focused our efforts on CD320, an LDL-domain containing receptor which is responsible for the uptake of Vitamin B12 (cobalamin) in normal and cancer cells [5]
- CD320 is overexpressed in cancer to meet increased demands for cobalamin [6]

TCPP measurement by flow cytometry



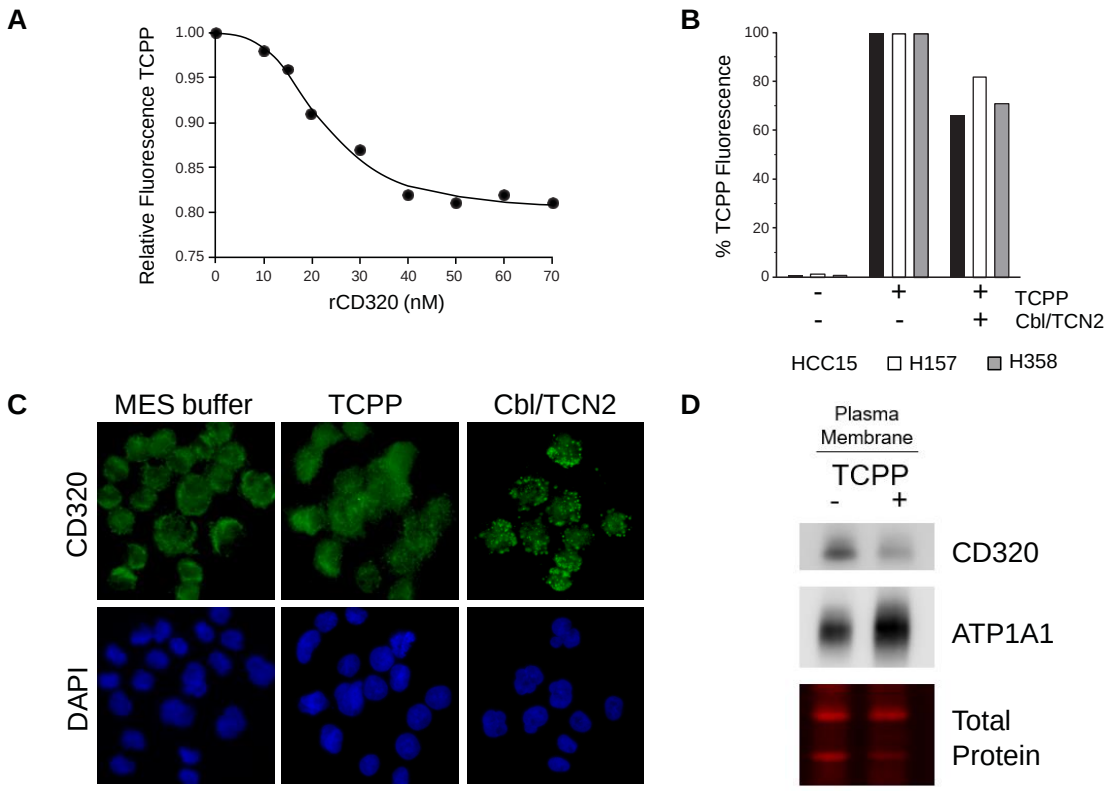
A flow cytometry assay was designed to determine TCPP uptake (A). Studies were performed to find the optimum uptake conditions (B, C) in HCC15 cells. Various lung, prostate, and breast cancer cell lines incorporated TCPP (D, E, F).

TCPP enters the cell via clathrin-mediated endocytosis



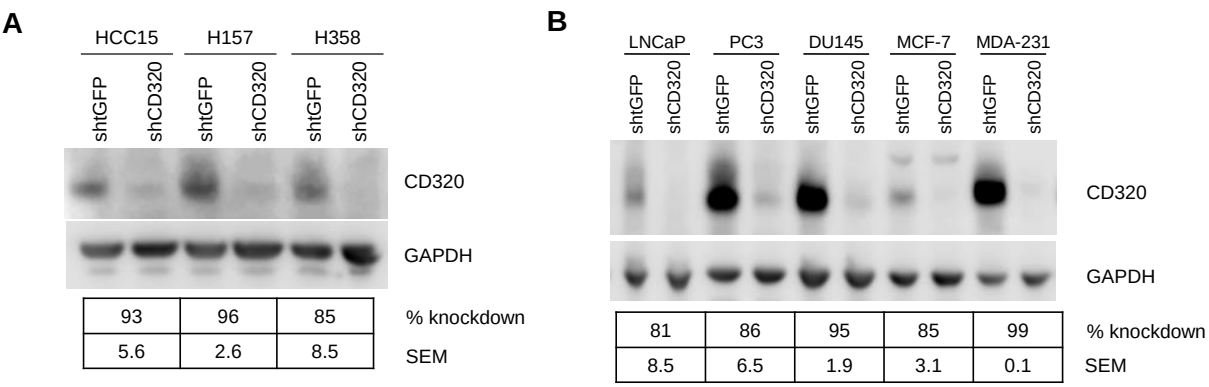
Cells were treated with general inhibitors of endocytosis (A), or inhibitors of specific endocytotic pathways (B, C, D, E) and measured for TCPP incorporation. All inhibitors of specific pathways were verified by measuring their inhibition activity in cell-based ELISA assays. CP = chlorpromazine, a specific inhibitor of clathrin endocytosis. (C, D) EIPA and ROT (rottlerin), inhibitors of clathrin-caveolin independent endocytosis. (E) Filipin, an inhibitor of caveolin-dependent endocytosis. * $p<0.05$ compared to DMSO

CD320 interacts with TCPP



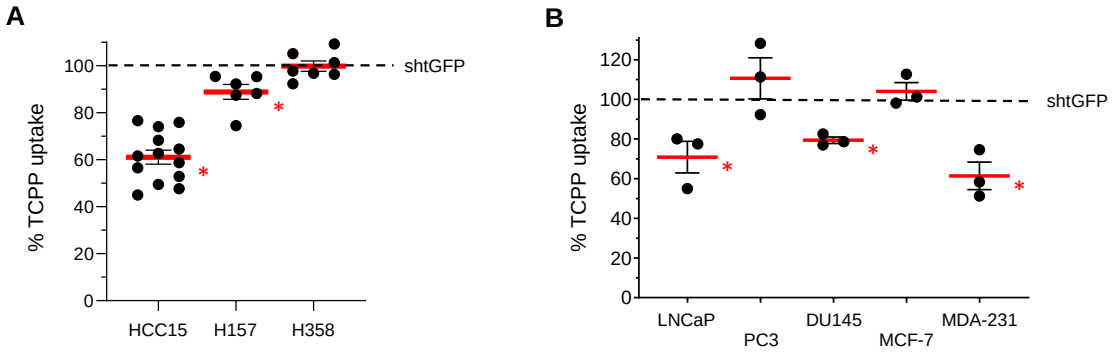
(A) Recombinant CD320 affected TCPP fluorescence, which allowed for a K_D determination of 42nm between CD320 and TCPP. (B) CD320's native ligand, transcobalamin II/ cobalamin (Cbl/TCN2), inhibited TCPP uptake in live cells. (C) Immuno-fluorescence microscopy and (D) sub-cellular fractionation show changes in CD320 localization upon TCPP exposure in HCC15 cells.

CD320 knockdown in multiple cell lines



Cells were infected with lentiviruses encoding shRNAs to CD320 or GFP (control). CD320 protein levels were assessed by western blot (A, B). GAPDH served as a loading control.

CD320 knockdown inhibits TCPP uptake in multiple but not all cancer cell lines



Cells were infected with lentiviruses encoding shRNAs to CD320 or GFP (control). TCPP uptake was measured by flow cytometry (A, B). * $p<0.05$ compared to shtGFP

Conclusions

- TCPP is differentially incorporated into diverse cancer cell lines
- TCPP is incorporated into cells by a clathrin-dependent endocytotic pathway
- Recombinant CD320 receptor binds to TCPP with a K_D of 42nm
- TCPP incorporation into cancer cells is inhibited by CD320's natural ligand Cbl/TCN2
- CD320 sub-cellular localization is altered upon TCPP exposure
- Silencing CD320 expression inhibits TCPP uptake in multiple cell lines but some cell lines are unaffected, suggesting that other receptors or mechanisms play a role in TCPP uptake

References

1. Rassmussen-Taxdal et al. (1955) Cancer 8, 78-81
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Porphyrins have been used successfully to treat and diagnose cancer, yet the mechanism of how porphyrins are selectively taken up and how they are retained by cancer cells compared to other cells remains poorly understood. Knowledge about the cellular uptake and retention mechanisms of porphyrins can be used to design more effective porphyrin-based diagnostics and therapeutics. We designed a flow cytometry assay to measure the cellular incorporation of meso-tetra (4-carboxyphenyl) porphyrin (TCPP) into cancer cell lines. We choose to study TCPP because it is currently used in a diagnostic assay for the early detection on lung cancer (1). Cell and molecular biology approaches show that TCPP enters cancer cells by a clathrin-mediated-endocytosis pathway. The LDL receptor, previously implicated in porphyrin uptake (2), only contributed modestly to TCPP uptake. In search of alternative TCPP entry mechanisms, we found that CD320, the cellular receptor for cobalamin/transcobalamin II (Cbl/TCN2) (3), bound strongly to TCPP (KD = 42 nM). Short hairpin RNA-mediated knockdown of CD320 resulted in up to 40% decrease in TCPP uptake in cell lines derived from lung, breast, and prostate cancer. The functional cellular uptake studies, combined with the results of the binding assay and other immunofluorescence microscopy studies, support our hypothesis that TCPP binds to CD320 and uses this receptor to enter cancer cells. Given that cancer cells over express CD320 (4), our research provides a reason for why TCPP accumulates in cancer cells.

REFERENCES

1. Patriquin et al. (2015) Journal of Thoracic Oncology 10, 1311–1318
2. Nakajima et al. (1995) Cancer Letters 92, 113–118
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