#P1205 Porphyrin uptake in lung cancer cells by dynamin-mediated endocytosis: a novel marker of dysregulated endocytosis in cancer

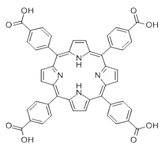
GREEHEY CHILDREN'S CANCER RESEARCH INSTITUTE
UT HEALTH SCIENCE CENTER® SAN ANTONIO

David J. Elzi[1], Peter Fatland[1], Bijal Karia[2], Moises Iza[1], Alexander Pertsemlidis[1], and Vivienne I. Rebel[1]

[1]Greehey Children's Cancer Research Institute, The University of Texas Health Sciences Center at San Antonio, San Antonio, TX 78229 and [2] BioAffinity Technologies Inc., San Antonio TX 78249

Abstract

Porphyrins are dynamic molecules involved in many biological processes, including oxygen and electron transport. Porphyrins have also been known to incorporate more readily into cancer cells compared to non-cancerous cells, and this property of porphyrins is being explored for diagnostic and therapeutic applications in cancer patients. The mechanism of how porphyrins are selectively incorporated into cancer cells is poorly understood, but the literature suggests that dysregulated endocytotic pathways may be a mechanism for porphyrin uptake. In addition, previous studies have suggested that porphyrins interact with the low-density lipoprotein receptor (LDLR) to incorporate into cells. We have examined the mechanism of incorporation of the porphyrin tetrakis(4-carboxyphenyl)porphyrin (TCPP) in lung cancer cells. We hypothesized that TCPP uptake in lung cancer cells is mediated by an endocytotic pathway through the LDLR. We analyzed TCPP uptake by flow cytometry in a panel of human lung cancer cell lines, including, HCC15, H157, and H358, while manipulating clathrin-dependent and independent endocytosis by chemical inhibitors. We found that sucrose and cold temperature moderately inhibited TCPP uptake in lung cancer cells. The dynamin inhibitor chlorpromazine, an antagonist of clathrin - mediated endocytosis, inhibited TCPP uptake by up to 80%, while the clathrin-independent endocytosis inhibitor filipin had no effect on TCPP uptake. To examine LDLR contribution on TCPP uptake, we pre-treated lung cancer cells with an anti-LDLR neutralizing antibody, which reduced TCPP uptake by 20%. Interestingly, TCPP uptake in human fibroblasts with or without functional LDLR showed no significant difference. These data suggest additional receptors and/or mechanisms are involved in TCPP uptake. In summary, our results show that the porphyrin TCPP is incorporated into lung cancer cells by a dynamin-mediated endocytotic pathway. Studies are underway to discover additional mediators of porphyrin uptake in these cancer cells. TCPP may provide a novel marker for monitoring dysregulated endocytosis in lung cancer and other cancers.

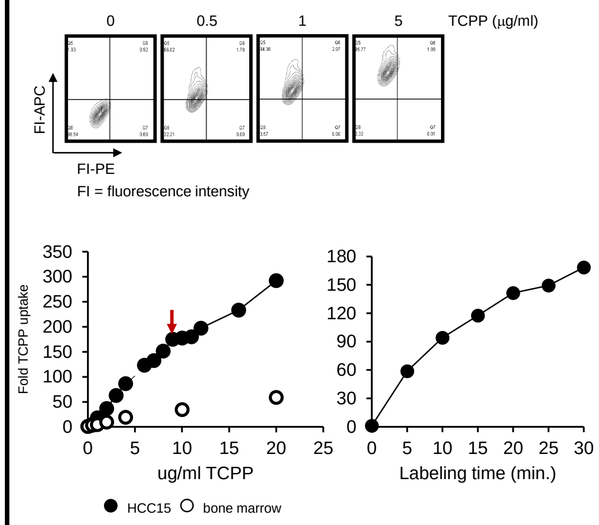


meso-Tetra(4-carboxyphenyl)porphine (TCPP)

Introduction

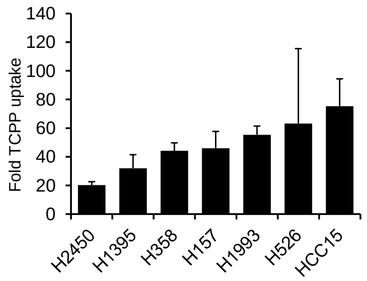
- Lung cancer is the number one cancer killer in men and women in the US
- Non-invasive diagnostic tests for early detection of lung cancer are needed to supplement low-dose computed tomography screening, which has a high false positive rate and often results in invasive procedures to validate cancer diagnosis
- Lung sputum can be collected and stained with the porphyrin TCPP, which selectively incorporates into cancer cells. TCPP incorporation can be monitored by its distinct fluorescence signature (Patriquin L et. al. J Thorac Oncol, 10, 1311-18, 2015.)
- The mechanism of how TCPP incorporates into cells and its specificity for cancer cells is unknown
- Previous research in porphyrin uptake has suggested the involvement of the LDL receptor in porphyrin selective incorporation into cancer cells
- Endocytosis is deregulated in cancer cells
- We want to examine the involvement of the LDL receptor and endocytosis in TCPP uptake in lung cancer cells

TCPP measurement by flow cytometry



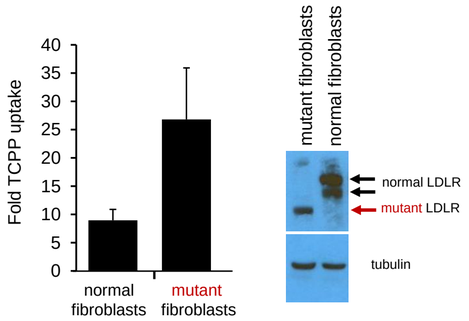
A flow cytometry assay was developed to monitor TCPP uptake in lung cancer cells. Optimum concentration and labeling times of TCPP were determined.

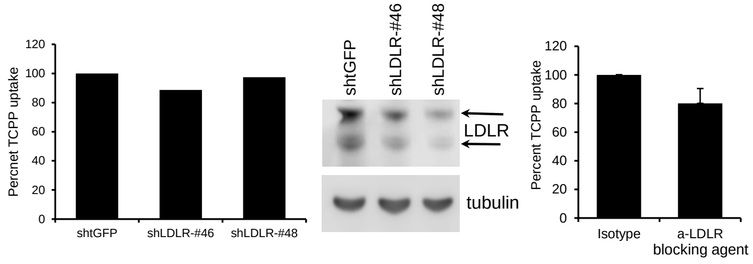
TCPP uptake varies among different lung cancer cell lines



A panel of lung cancer cell lines were assayed for TCPP uptake by flow cytometry. Cells were incubated for 10 minutes at 37°C with 9μg/ml TCPP.

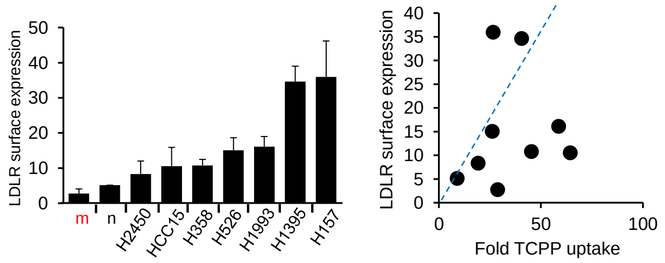
LDLR is not essential for TCPP uptake into the cell





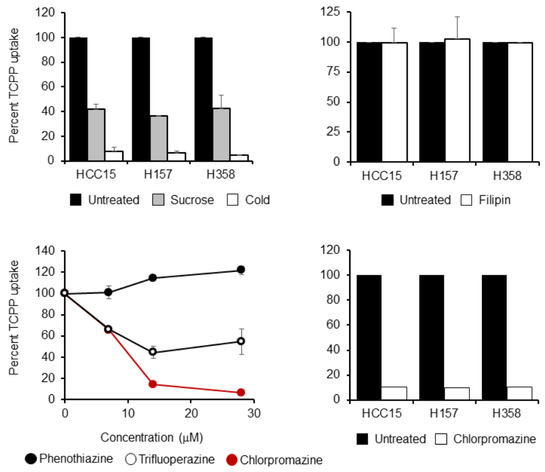
LDLR functional activity and expression were attenuated by genetic methods (human fibroblasts with mutant LDLR), shRNA to LDLR, and anti-LDLR blocking antibody. TCPP uptake was measured by flow cytometry.

LDLR surface expression does not correlate with TCPP uptake in lung cancer cells



TCPP uptake was measured by flow cytometry. The correlation between LDLR surface expression and TCPP uptake is shown.

TCPP enters the cell through clathrin-mediated endocytosis



Lung cancer cells were treated with indicated inhibitors of endocytosis. TCPP uptake was measured by flow cytometry.

Conclusion

- TCPP is differentially incorporated into lung cancer cells
- Reducing LDLR activity by genetic or antibody methods modestly reduce TCPP uptake
- LDLR surface expression does not correlate with TCPP uptake, suggesting alternative pathways for TCPP uptake
- TCPP uptake in cells is mediated by endocytosis. This uptake is inhibited by clathrin/dynamin inhibition, but not caveolin inhibition
- TCPP may represent a novel indicator for endocytosis in cancer cells