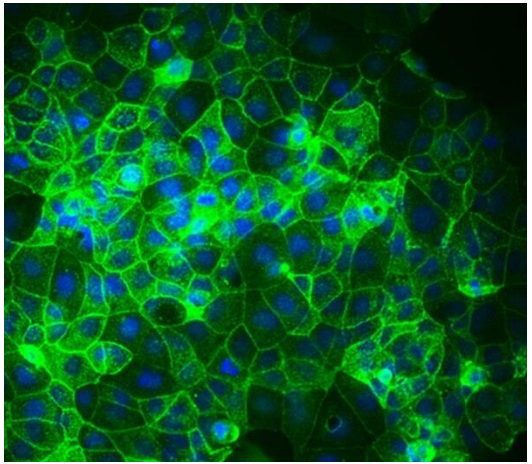
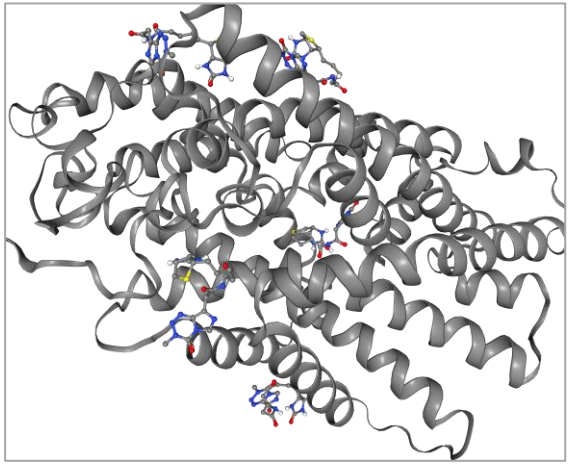
# Study focusing on human Sodium-dependent Multivitamin Transporter (hSMVT) and its application in cancer cell targeting



*Caco-2 Cancer Cell Line*
*Source. Sigma Aldrich*



*CB Dock software results showing docking between Biotin-Cancer Drug conjugate and SMVT protein model (Mhp1)*

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*Los Alamitos High School*

# Abstract

Colon cancer is the third leading cause of cancer related deaths with over 940,000 deaths reported worldwide in 2020. The overexpression of certain membrane bound proteins, such as Sodium-dependent Multivitamin Transporter (SMVT), allow colonic cancer cells to be targeted. My project involves studying the SMVT protein overexpression and designing a biotin cancer drug (temozolomide) conjugate for cancer cell targeting. I studied SMVT regulation using proteasomal (Lactacystin) and lysosomal (Bafilomycin) pathway inhibitors to analyze degradation pathways of SMVT. The results of my experiments show higher SMVT expression after cells were treated with both inhibitors. Since cancer cells often have several mutations which affect proteins, experiments to learn about SMVT functionality in cancer cells were conducted. The results show an increase in biotin uptake after the cells were treated with either inhibitor, indicating that the overexpressed SMVT are functional. Since biotin conjugates have been shown to be transported by SMVT across cell membrane, I created a model for a biotin conjugate using the cancer drug temozolomide. I used protein model Mhp1, homologous to SMVT, and the biotinylated temozolomide to test the docking. I found 5 possible binding sites where the biotinylated conjugate could bind, with the strongest dock having a binding energy of -8.9 kCal/mole. My project provide important insights into SMVT protein expression in the context of drug targeting for cancer therapy. For future work, I plan to extend this project as a framework of drug targeting for other forms of cancer which also express the SMVT protein.

# Introduction

- Globally, colon cancer caused 940,000 deaths in 2020 (Xi and Xu, 2021). In the US, colon cancer is the 3rd leading cause of cancer-related deaths in men and in women estd. ~54,000 deaths in 2022 (www.cancer.org)

- Sodium dependent multivitamin transporter (SMVT) transports vitamin biotin (B7), the vitamin pantothenic acid (B5), and lipoic acid across the cell membrane (Wang et al. 1999, De Carvalho and Quick 2011)

- SMVT overexpression has been shown in colon cancer cells (Said et al.,1998), providing a possible route for increased uptake and targeted distribution of cancer drugs

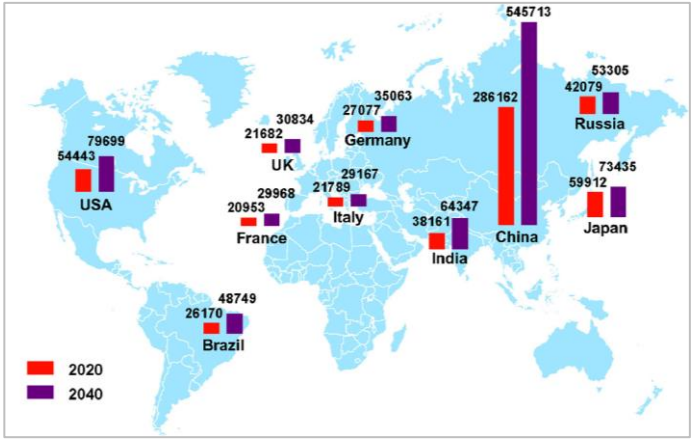



Figure 1. Colon cancer related deaths top 10 countries with highest incident cases in 2020 and projections for 2040. *Source: https://www.sciencedirect.com/*

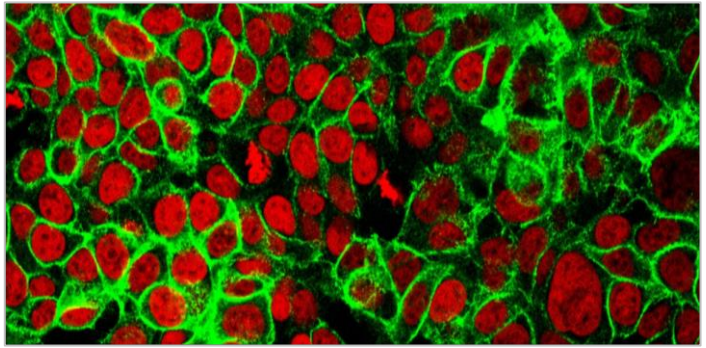


Figure 2. Human colon cancer cells with the cell nuclei-stained red. *Source: https://news.harvard.edu/*

# Background Research

## SMVT Function

- Biotin (B7) and pantothenic acid (B5) which are essential vitamins are not synthesized within the body. SMVT plays an important role by transporting B7 and B5 in the body to maintain bodily equilibrium.

## SMVT Regulation and Degradation Pathways

- Ubiquitination is a modification in which a ubiquitin molecule is attached to a protein. Ubiquitin destabilize and shorten the half life of proteins. Studies show evasion of this process leads to longer half life and greater protein expression (Abu Ahmad et al., 2021).

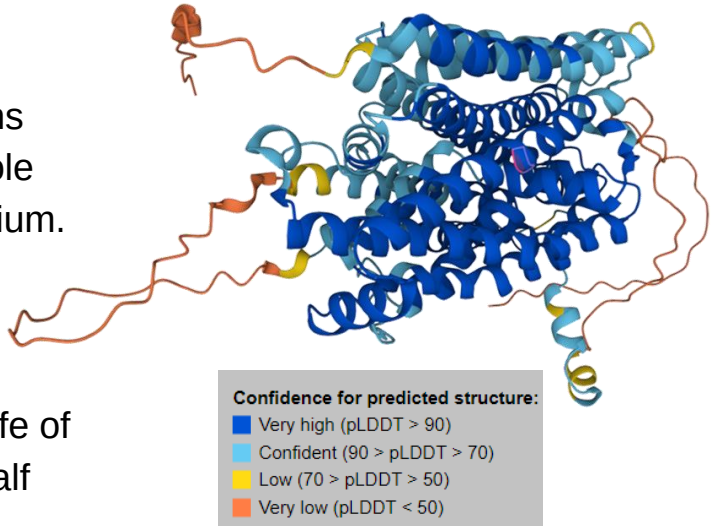



Figure 3. SMVT (SLC5A6) predicted structure
Source. Alphafold project. Structure version 2

## Drug Targeting with SMVT

- Multiple studies show biotinylated conjugates have potential for drug delivery: Biotinylated conjugate acyclovir has been studied with the purpose of increasing cellular absorption by targeting SMVT on the cornea for the treatment of herpetic keratitis (Vadlapudi et al. 2013)
- Cancer cells need larger supplies of certain vitamins, so certain vitamin receptors like SMVT involved in vitamin uptake are overexpressed. Cytotoxin with conjugated biotin as a targeting agent achieved greatest anti-tumor activity among the different vitamin conjugates (Russell-Jones et al. 2004).

# Hypothesis

I hypothesize that SMVT protein is functional and subject to ubiquitin mediated degradation pathway in cancer cells.

I also hypothesize biotinylated (vitamin b7) cancer drug, temozolomide, will bind with SMVT thus allowing greater uptake of the temozolomide within the cancer cell line Caco-2.

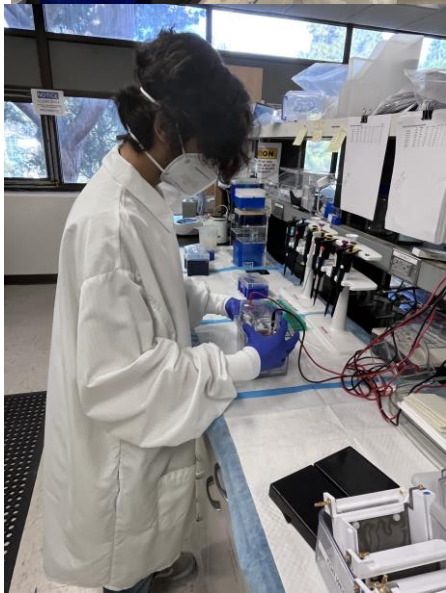
Objectives:

1. To understand the SMVT transporter protein expression by evaluating proteasomal and lysosomal degradation pathways.

2. Create a biotin and drug conjugate for SMVT protein to target colon cancer cells.

# Materials

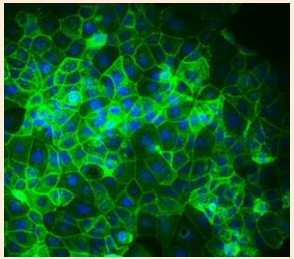
- Caco-2 Cell Line (Sigma-Aldrich 86010202-1VL)
- Proteasomal Inhibitor Lactacystin (Sigma-Aldrich L6785)
- Lysosomal Inhibitor Bafilomycin (BafA1) (Sigma-Aldrich B1793)
- Vitamin B7 Biotin (Sigma-Aldrich B1116000)
- Sodium Dodecyl Sulfate- Poly Acrylamide Gel Electrophoresis (SDS- PAGE) Buffer
- Western Blotting Buffer
- Krebs Ringer (KR) Buffer (so cell will not lyse, keeps osmotic pressure balance)
- Sodium Hydroxide (NaOH), Hydrochloric Acid (HCL)
- Eagle's Minimum Essential Medium (ENEM) Media
- Spectrophotometer
- Plates for cell, Oven
- Modeling and Visualization Software: Phyre2, Avogadro, CB Dock, Image Studio



# Methods

## 1. Understanding SMVT Protein Expression

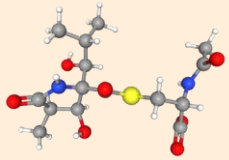
### Monitoring Cell Lines



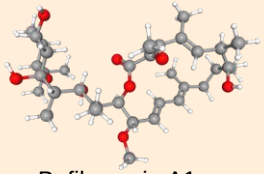
Caco 2 (Source Sigma-Aldrich)

### Variables

**Regulation Test**

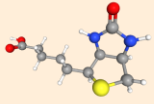


Lactacystin



Bafilomycin A1

**Function Test**



Biotin

### Methods to Demonstrate SMVT pathways

- SDS Page
- Western Blotting
- Cellular Protein Estimation
- Quantification of Protein Band Intensity

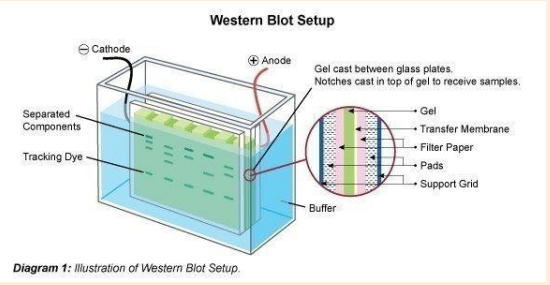
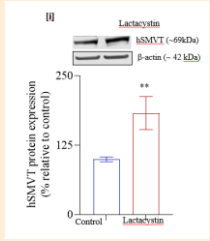



*Diagram 1: Illustration of Western Blot Setup.*

### Observation Parameters




**Protein expression**

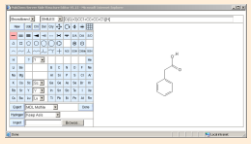
- Proteasomal and Lysosomal degradation pathway

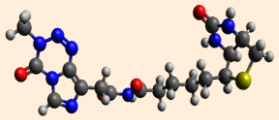
**Protein function**

- Biotin uptake

## 2. Molecular Modeling of Biotin and Cancer Drug Temozolomide Conjugate

### Model Preparation for Biotin and Drug Conjugate

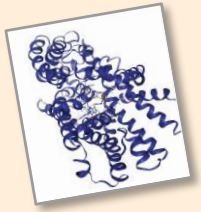




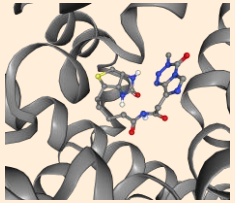
- **PubChem Sketcher Tool**
- **Avogadro software**

### SMVT Homology Modeling

Used Sequence of SMVT homologous protein Mhp1 from NCBI, translated to a 3D structure using **Phyre2**, and converted to PDB file



### Explore different SMVT binding sites with biotin-drug conjugate



| Pose | Binding Energy | Cavity Size | Center | | | Size | | |
|---|---|---|---|---|---|---|---|---|
| | kCal/Mole | | x | y | z | x | y | z |
| 1 | -8.9 | 2400 | 3 | 5 | -28 | 26 | 26 | 30 |
| 2 | -7.4 | 364 | 2 | 33 | -23 | 26 | 26 | 26 |
| 3 | -6.6 | 230 | -8 | 29 | -8 | 26 | 26 | 26 |
| 4 | -6.3 | 320 | 10 | 13 | -42 | 26 | 26 | 26 |
| 5 | -6.0 | 202 | 10 | 24 | 4 | 26 | 26 | 26 |

**CB Dock software**

# Methods (contd.)

1) **Caco 2 cells lines** were used as the **monitoring cell line** in the SMVT protein regulation and function experiments.
2) **SMVT Regulation experiments** – These experiments were conducted using proteasomal inhibitor Lactacystin and Lysosomal inhibitor BafA1 (Bafilomycin). Protein degradation pathways were observed using SDS Page and Western Blotting methods. After protein was isolated, denatured, and SDS applied, protein was run through electrophoresis. After electrophoresis, western blotting with primary and secondary antibodies was done to isolate protein of interest. Gel scanned after western blotting and analyzed using Image Studio software.
3) **SMVT Function experiments**- These experiments to determine SMVT functionality through biotin uptake were conducted using proteasomal inhibitor Lactacystin and Lysosomal inhibitor BafA1 (Bafilomycin). The uptake of biotin was estimated using labeled biotin and scintillation counter.
4) **Molecular modeling using software** - A molecular model of biotin and cancer drug temozolomide conjugate was created using PubChem sketcher tool. The visualization software Avogadro was used to visualize the biotin-drug conjugate.
5) **SMVT homologous protein Mhp1 sequence** was used from NCBI, translated to a 3D structure using **Phyre2**, and converted to **PDB file**.
6) **Using the PDB file**, I used the **CB Dock Software** to explore different SMVT binding sites with biotin-drug conjugate model.

**Understanding SMVT Protein Expression**

Steps 1-3: The lab experiment portion of my project was conducted by me under supervision in a regulated lab at the University of California, Irvine.

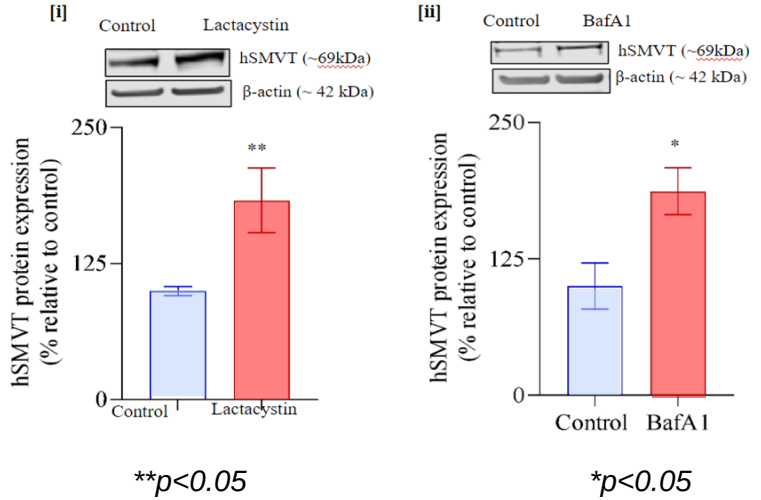
**Molecular Modeling of Biotin and Cancer Drug Temozolomide Conjugate**

Steps 4-6: This portion of my project was conducted by me using desktop and online molecular modeling software.

# Results

## SMVT Expression Tests

- Results show SMVT is subject to ubiquitin mediated degradation pathways or ubiquitination
- For proteasomal inhibitor, Lactacystin, greater SMVT expression is observed in comparison to control
  - Statistically significant results with a P-value < 0.05
- For lysosomal inhibitor, BafA1 (Bafilomycin), greater SMVT expression is observed in comparison to control
  - Statistically significant results with a P-value < 0.05




***p<0.05* **p<0.05*

Figure 4. SMVT expression results using Lactacystin and Bafilomycin

| Control | Lactacystin |
|---|---|
| 84 | 198 |
| 99 | 290 |
| 100 | 116 |
| 109 | 155 |
| 106 | 155 |

| Control | BafA1 |
|---|---|
| 57 | 254 |
| 169 | 192 |
| 68 | 135 |
| 129 | 208 |
| 77 | 146 |

# Results (Cont.)

**SMVT Function Tests**

- SMVT is functional as shown by greater uptake of biotin
- For proteasomal inhibitor, Lactacystin, greater biotin uptake is observed in comparison to control
  - Statistically significant results with a P-value < 0.01
- For lysosomal inhibitor, BafA1, greater biotin uptake is observed in comparison to control
  - Statistically significant results with a P-value < 0.01

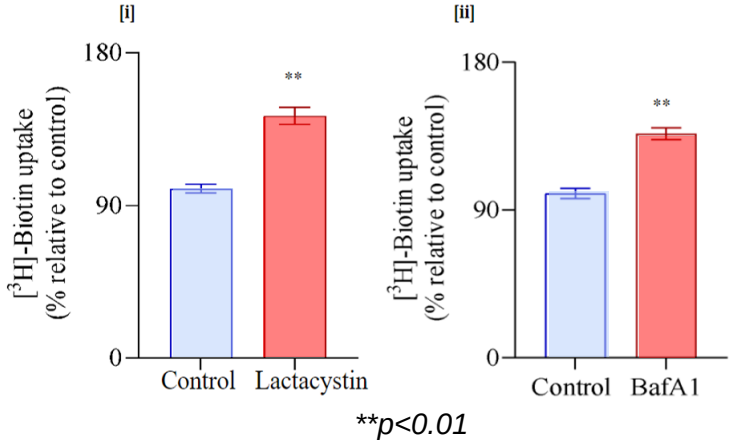



Figure 5. SMVT function results using Lactacystin and Bafilomycin

| Control | Lactacystin |
|---|---|
| 113 | 114 |
| 96 | 137 |
| 91 | 167 |
| 100 | 140 |
| 85 | 167 |
| 100 | 143 |
| 115 | 163 |
| 100 | 158 |

| Control | BafA1 |
|---|---|
| 107 | 144 |
| 110 | 131 |
| 83 | 127 |
| 100 | 134 |
| 107 | 137 |
| 96 | 124 |
| 98 | 156 |
| 100 | 139 |

# Results (Cont.)

## Creating Biotin Drug Conjugate

- 2D and 3D models for NHS Biotin and NHS Biotin Temozolomide Conjugate (biotinylated temozolomide) created with PubChem sketcher and Avogadro

*Source. https://pubchem.ncbi.nlm.nih.gov//edit3/index.html*

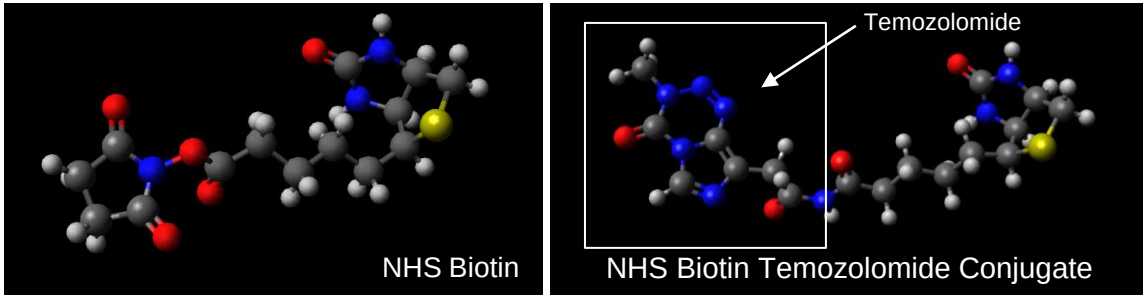



*Figure 6. 3D model for visualization of Biotin Drug Conjugate*

## Docking Test Results for SMVT and Biotin Drug Conjugate

- Sequence of homologous protein SMVT(Mhp1) available on NCBI, translated to a 3D structure using Phyre2, and converted to PDB file
- Used CB Dock to test docking between conjugate and Mhp1

*Source. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7471403/*

*Table 1. Comparison of Docking Sites*

| Pose | Binding Energy | Cavity Size | Center | | | Size | | |
|---|---|---|---|---|---|---|---|---|
| | kCal/Mole | CB Unit | x | y | z | x | y | z |
| Strongest → 1 | -8.9 | 2400 | 3 | 5 | -28 | 26 | 26 | 35 |
| 2 | -7.4 | 354 | 2 | 33 | -23 | 26 | 26 | 26 |
| 3 | -6.6 | 230 | -8 | 29 | -8 | 26 | 26 | 26 |
| 4 | -6.3 | 320 | 10 | 13 | -42 | 26 | 26 | 26 |
| Weakest → 5 | -6.0 | 202 | 10 | 24 | 4 | 26 | 26 | 26 |

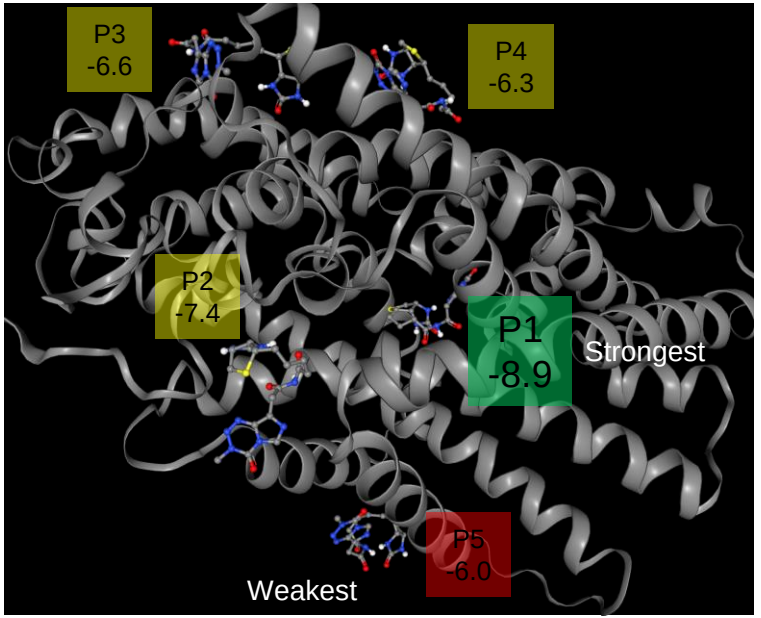



*Figure 7. Visualization of docking results showing 5 binding sites*

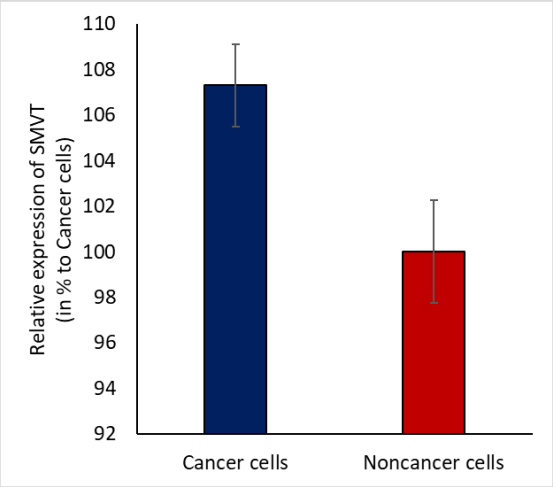
# Discussion

- Relative SMVT gene expression in cancer cells is higher with a statistically significant difference demonstrating how SMVT is overexpressed. (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183749)

- When membrane proteins are ubiquitinated, they can follow either the proteasomal or lysosomal pathway of degradation. Since both degradation pathways were observed, it is certain that the SMVT protein is ubiquitinated.

- Biotin uptake increased after cells treated with both inhibitors, indicating that the overexpressed proteins are functional forms of the SMVT.

- Docking results show that the biotin drug conjugate has 5 sites where it can possibly bind, transforming temozolomide delivery to a specific form of delivery
  - Strongest dock has a binding energy of -8.9 kCal/mole
  - Weakest dock has a binding energy of -6.0 kCal/mole




*p<0.05*

Figure 8. Relative SLC5A6 (SMVT gene) expression

# Conclusion

- SMVT is subject to ubiquitination therefore proving protein overexpression is not due to an evasion of ubiquitination
- Overexpression of the SMVT gene is the reason that the SMVT protein is expressed at a higher level in cancer cells
- Colonic cancer cells produce a functional form of SMVT, allowing it to be a target for drug treatment
- Through modeling, I demonstrated Biotinylated Temozolomide is able to bind with Mhp1, the model used to represent SMVT.

# Reflection/ Application

- Importance of SMVT protein: SMVT plays an important role in maintaining bodily equilibrium by bringing biotin into the cell where it is used to help metabolize energy.

- Limitation of the study: I used bioinformatics and computational structure prediction models to overcome some of the limitations of lab experiments. I did not have the equipment and resources to conjugate the biotin and drug, then verify if the two were bound together. Data for noncancerous cells was taken from literature review because experiments were not conducted with noncancerous cells.

- Broader application of this research: SMVT is widely expressed in many tissues including the intestines (small and large), kidney, heart, lung and brain. A similar approach and framework for drug targeting can be used for application in a broader context for other types of cancer in organs where SMVT is over expressed.

- Next Steps: Some next steps are i. experiments to quantify SMVT expression in healthy cells, ii. create biotinylated temozolomide and monitor expression in caco-2 cells in comparison to temozolomide alone, and iii. experiment to isolate RNA of cancer cells to verify gene expression study.

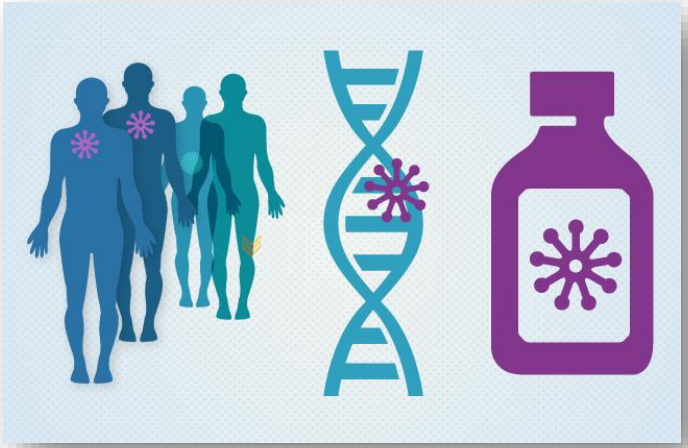


Figure 9. Targeted therapy treats cancer by targeting proteins that control how cancer cells grow, divide, and spread. *Image Credit: National Cancer Institute*

# References Cited/ Prior Research

Acknowledgement of Prior Research: The lab experiments conducted in the project are a subset of a larger project focusing on the regulation and function of SMVT in inflammatory bowel disease and biotin deficiency at UC Irvine. Although the same methods were used, modeling and application is independent of the larger project.

1) Abu Ahmad Y, Oknin-Vaisman A, Bitman-Lotan E, Orian A. From the Evasion of Degradation to Ubiquitin-Dependent Protein Stabilization. Cells. 2021 Sep 9;10(9):2374. doi: 10.3390/cells10092374. PMID: 34572023; PMCID: PMC8469536.

2) De Carvalho FD, Quick M: Surprising substrate versatility in SLC5A6: Na+-coupled I - transport by the human na+/multivitamin transporter (hSMVT). Journal of Biological Chemistry 286(1): 131-137, 2011

3) Russell-Jones G, McTavish K, McEwan J et al.: Vitamin-mediated targeting as a potential mechanism to increase drug uptake by tumours. Journal of Inorganic Biochemistry 98(10): 1625- 1633, 2004

4) Said H, Ortiz A, McCloud E et al.: Biotin uptake by human colonic epithelial NCM460 cells: A carrier- mediated process shared with pantothenic acid. American Journal of Physiology- Cell Physio. 275(44-5): 1998

5) Vadlapudi AD, Vadlapatla RK, Earla R et al.: Novel biotinylated lipid prodrugs of acyclovir for the treatment of herpetic keratitis (HK): Transporter recognition, tissue stability and antiviral activity. Pharmaceutical research 30(8): 2063, 2013

6) Wang H, Huang W, Fei YJ et al.: Human placental na+ dependent multivitamin transporter. cloning, functional expression, gene structure, chromosomal localization. Journal of Biol Chem 274(21):14875-14883, 1999

7) Yue Xi, Pengfei Xu, Global colorectal cancer burden in 2020 and projections to 2040,Translational Oncology,Volume 14, Issue 10,2021,101174, ISSN1936-5233, https://doi.org/10.1016/j.tranon.2021.101174.