# Metabolic Reprogramming and Clinical Behaviors of Cancer

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# Questions Interested in Answering

- What are the main causes of a cancer?
- Answer from the literature: genomic mutations
- But all chronic inflammation related diseases have just as many genomic mutations

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**Somatic Mutation, Genomic Variation, and Neurological Disease**

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*NATURE GENETICS* | LETTER

日本語要約

Genome-wide association identifies multiple ulcerative colitis susceptibility loci

nature genetics

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*NATURE GENETICS* | LETTER

日本語要約

Somatic mutations of the Parkinson's disease–associated gene *PARK2* in glioblastoma and other human malignancies

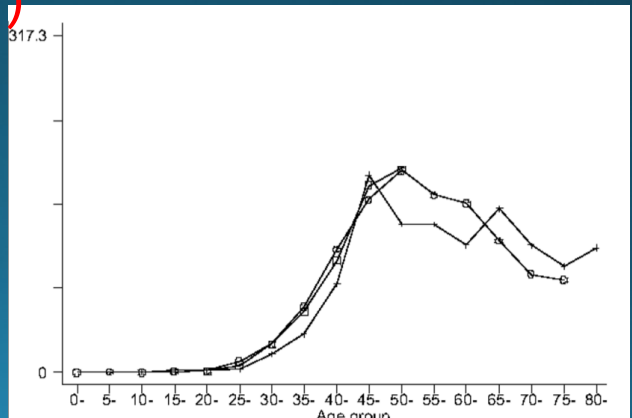
**rial genome mutations in diabetes].**

Hieronimus S, Vague P, Saunières A, Desnuelle C.

# Questions Interested in Answering

- What drives a cancer to metastasize?
- A popular assumption is: tumor growth runs out of space
- But cancer like melanoma starts to metastasize as soon as the tumor grows vertically while some other cancers can grow to substantial sizes without metastasis

- Why do cancer occurrence rates in general have a bell-shaped curve over age, and different cancers peak at different ages; some could be as early as 30+ (Testis cancer)



# Questions Interested in Answering

- What determine the malignance level of a cancer?
- The current literature has no answer.

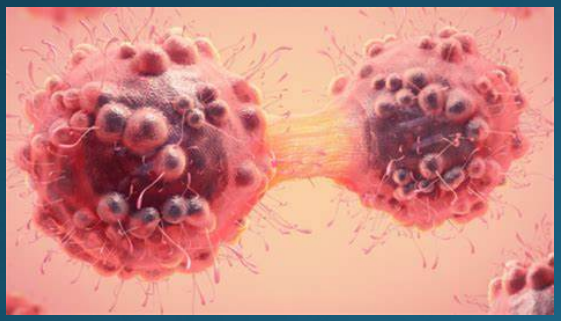
- I aim to show that some or possibly most of these and many other cancer related (fundamental) questions may be answerable through data mining and computational modeling!

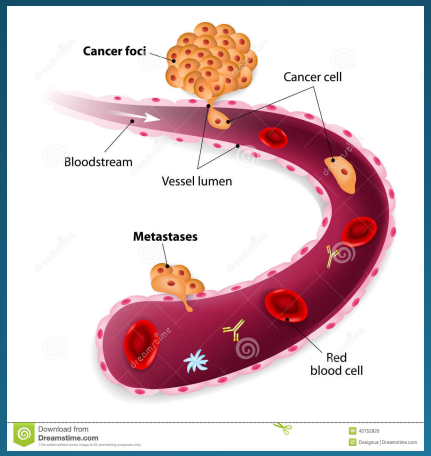
# What Defines a Cancer

- The predominant research and treatment efforts have been on stopping cell division and attacking cells with certain antigens
- Cancer actually has numerous other intrinsic characteristics: migration, metastasis, drug resistance, reduced blood level of sodium, cachexia …
- Very little has been established regarding the functional relationships among all these

# Main Question to Address Here

- Are all these clinical behaviors of cancer intrinsically linked through some unknown common drivers?







# From Data to Knowledge

- We have analyzed gene-expression data of over 10,000 cancer tissue samples and 10,000+ non-cancerous chronic inflammatory disease samples

- We study possible drivers of cancer initiation, development, metastasis and other clinical behaviors through data analyses and computational modeling

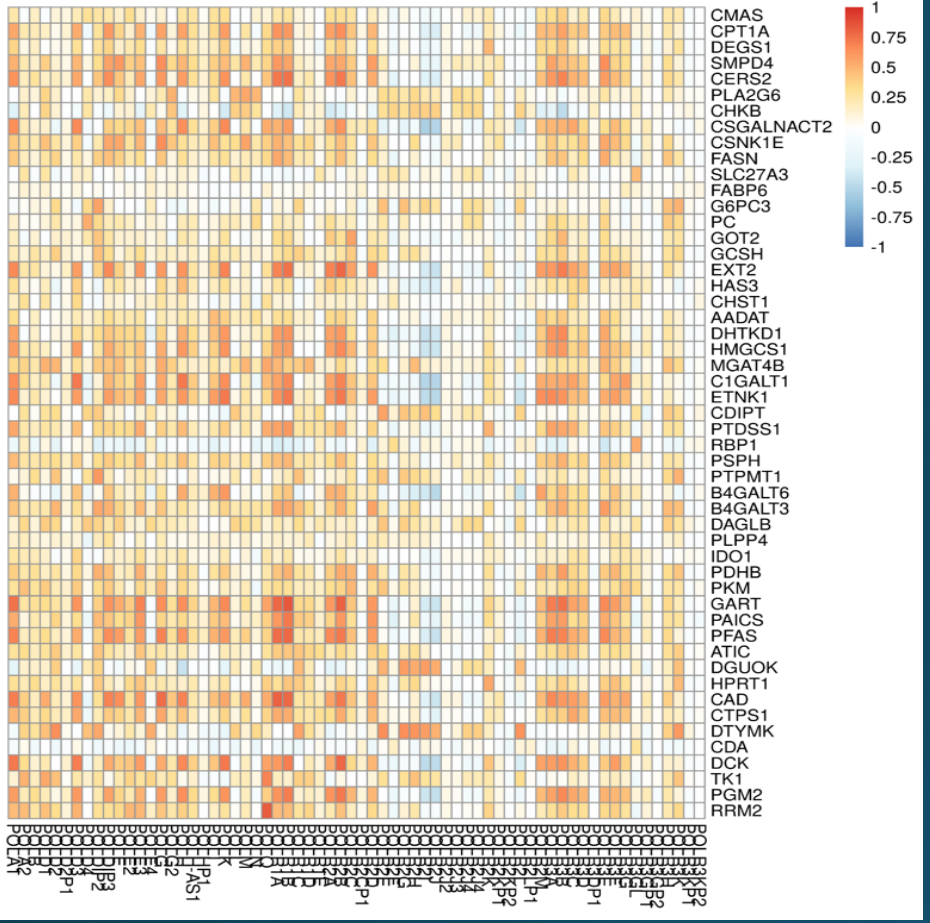
- We focus on fundamental balances and possible relationships between persistent disruption of such balances and disease development instead of detailed molecular pathways

# Cancer Metabolic Reprogramming

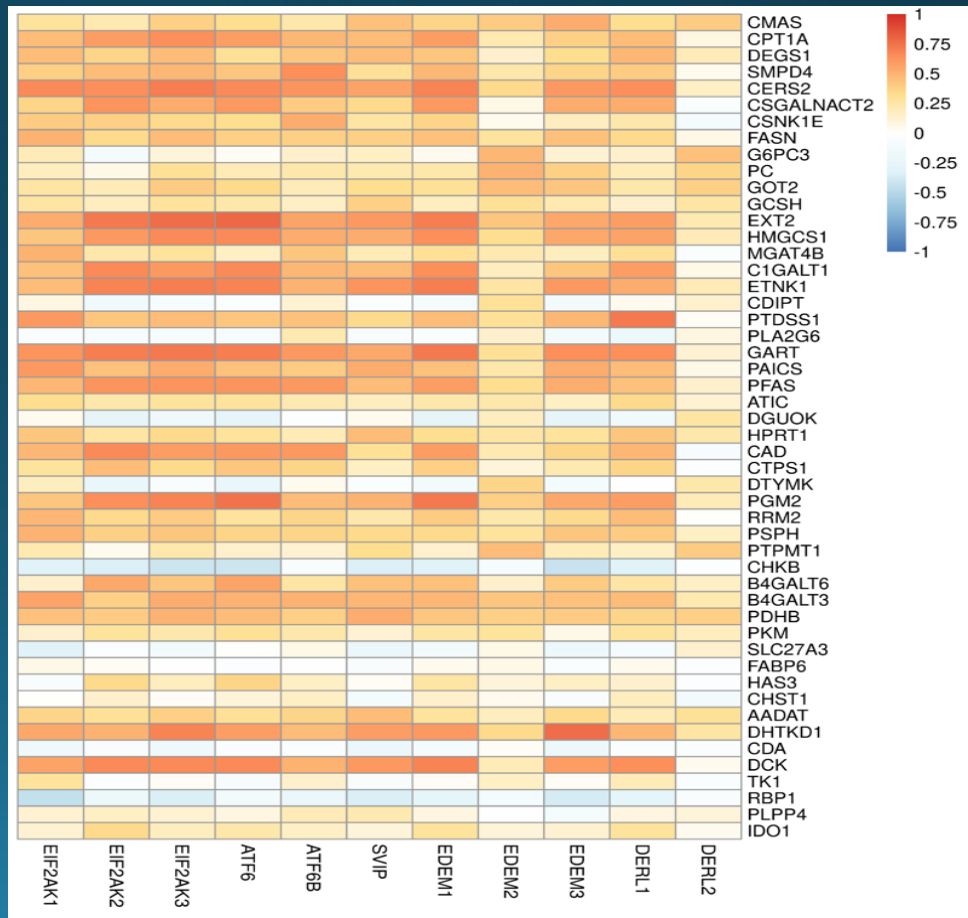
- Substantial changes take place in metabolisms in cancer
- Cancer tends to synthesize *de novo* nucleotides instead of uptake from circulation via the salvage pathway
- Cancer tends to inhibit urea cycle for removal of the waste, $NH_3$, of amino acid metabolism
- Cancer tends to considerably over-produce sialic acids and deploy them on cancer cell surface

# Cancer Metabolic Reprogramming

- Cancer tends to repress arginine synthesis, uptake and utilization
  - Arginine has by far the highest mutation rate among all amino acids
- Cancer tends to use an inefficient way to produce energy, namely fermentation instead of the normal, more efficient respiration process, called the Warburg effect
- And many more …
- All these are considered as changes selected in support of cell proliferation




Metabolic reprogramming is probably the result of stress, at least to a large degree



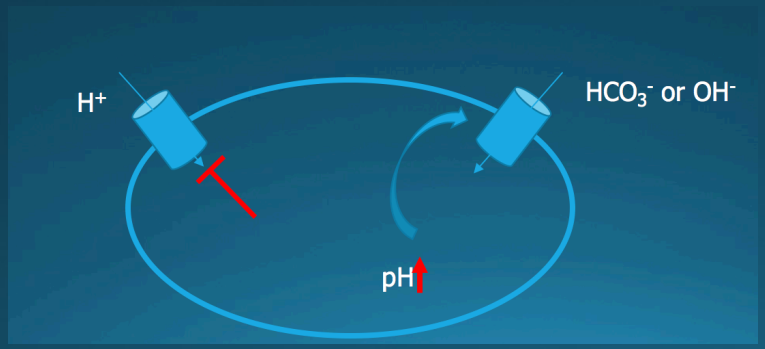

## Cell cycle genes

Metabolic changes correlate with stresses at least as strongly as with cell proliferation (breast cancer)

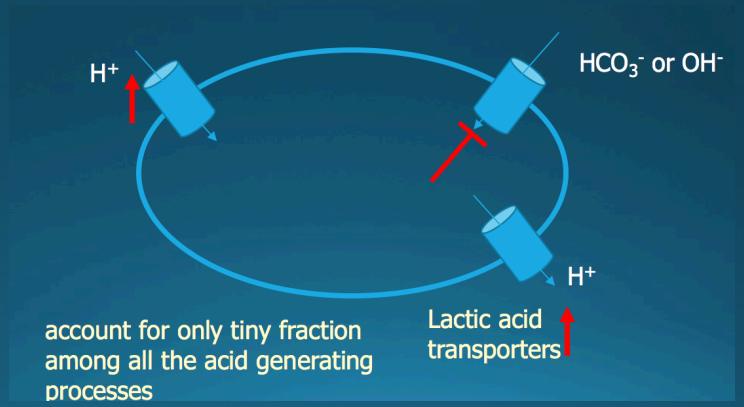
## Stress genes

# Intracellular pH in Cancer and Normal Cells

- Normal cells have acidic cytosolic pH (~6.8) and cancer & all proliferating cells have alkaline pH (7.2-7.5)




Normal proliferating cells

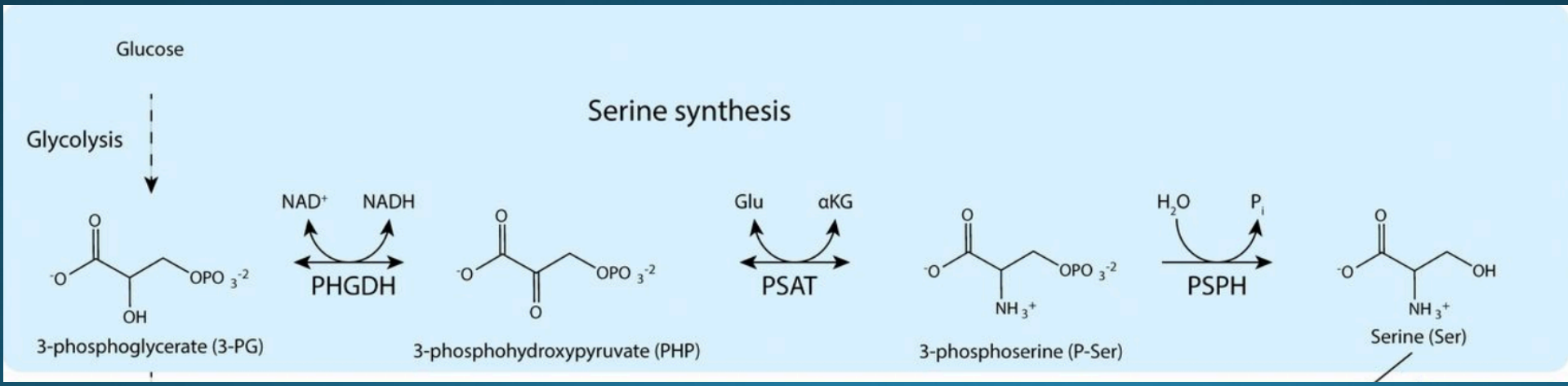



Cancer cells

… suggesting pH related stress

# Serine Synthesis in Cancer

- Compared to normal proliferating cells, cancer (tissue) cells substantially increase synthesis of amino acid serine rather than uptake it from circulation;
- and more malignant cancers tend to have higher levels of serine synthesis.
- A key function of serine in cancer is used for nucleotide synthesis



# Serine Synthesis in Cancer

- The overall synthesis reaction can be written as

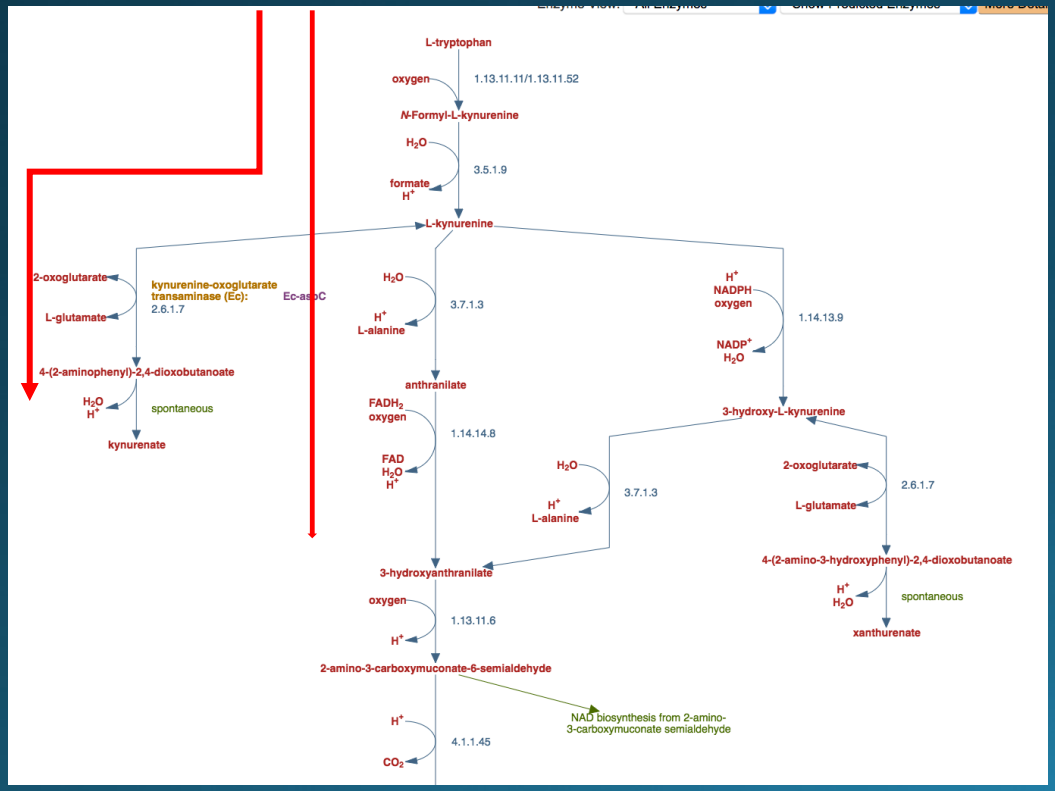
$$\text{glutamate} + 3\text{PG} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow 2\text{-oxoglutarate} + \text{P}_i + \text{serine} + \text{NADH} + \text{H}^+$$

- which produces one net proton. In comparison, serine uptake by SLC1A4/A5, the main transporters of serine, is pH neutral.

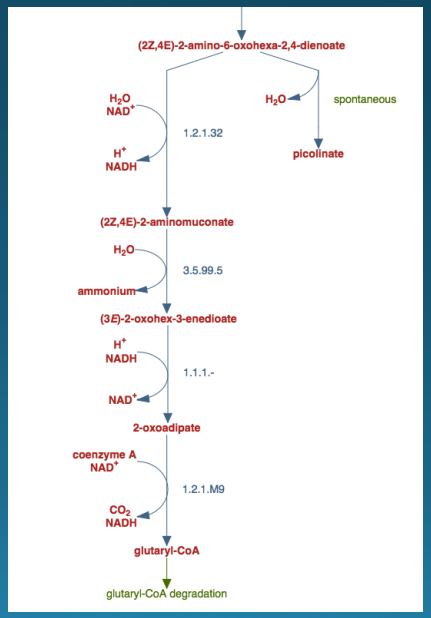
NOTE: When analyzing cancer data, NAD+ and NADH have to be analyzed separately rather than as one closed system as in normal cells.

# Tryptophan Degradation in Cancer

- Normal cells degrade tryptophan to acetyl-CoA via the following pathway but cancer only uses part of the pathway to produce kynurenine and 3-hydroxyanthrranliate



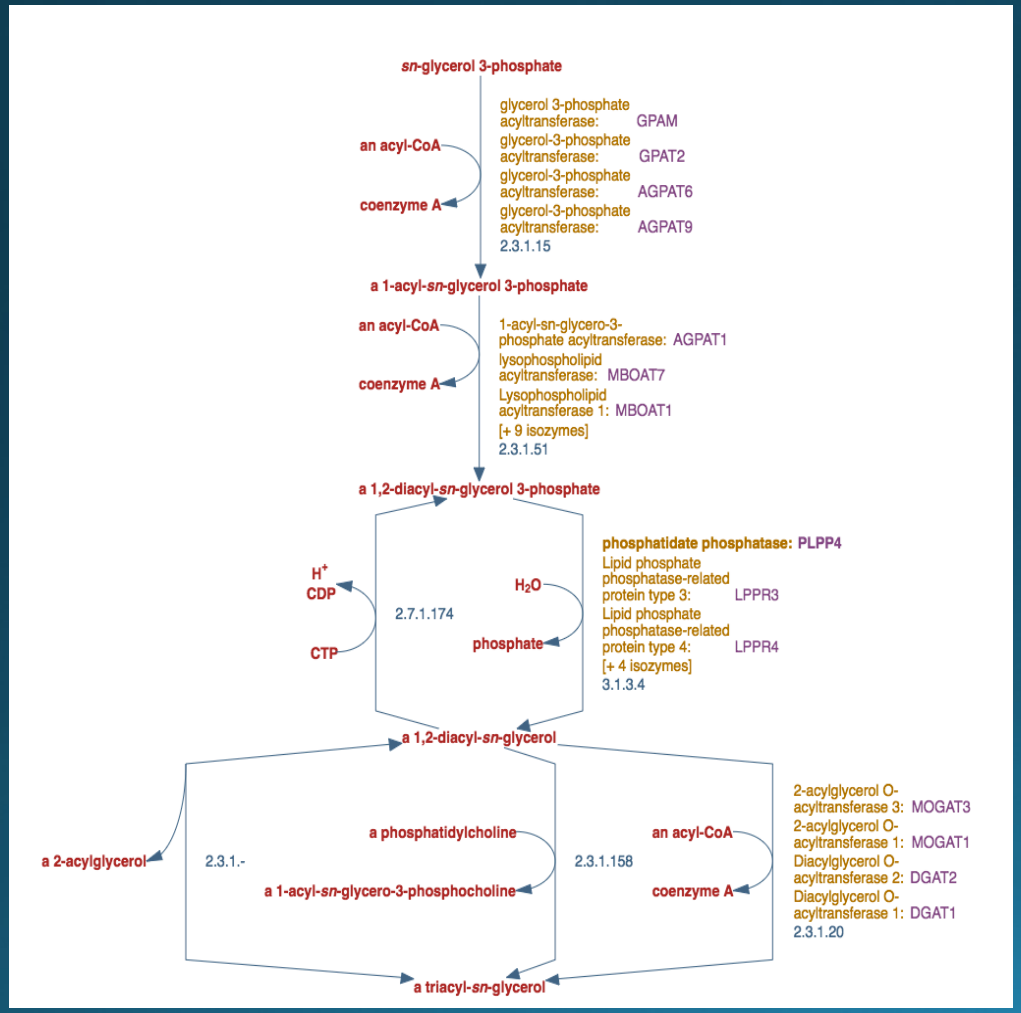

It produces the most number of protons

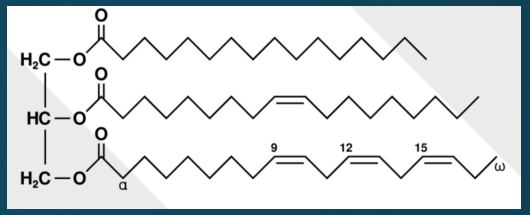



acidification

# Increased Triglyceride Synthesis

- Cancer increases the activity of triglyceride synthesis



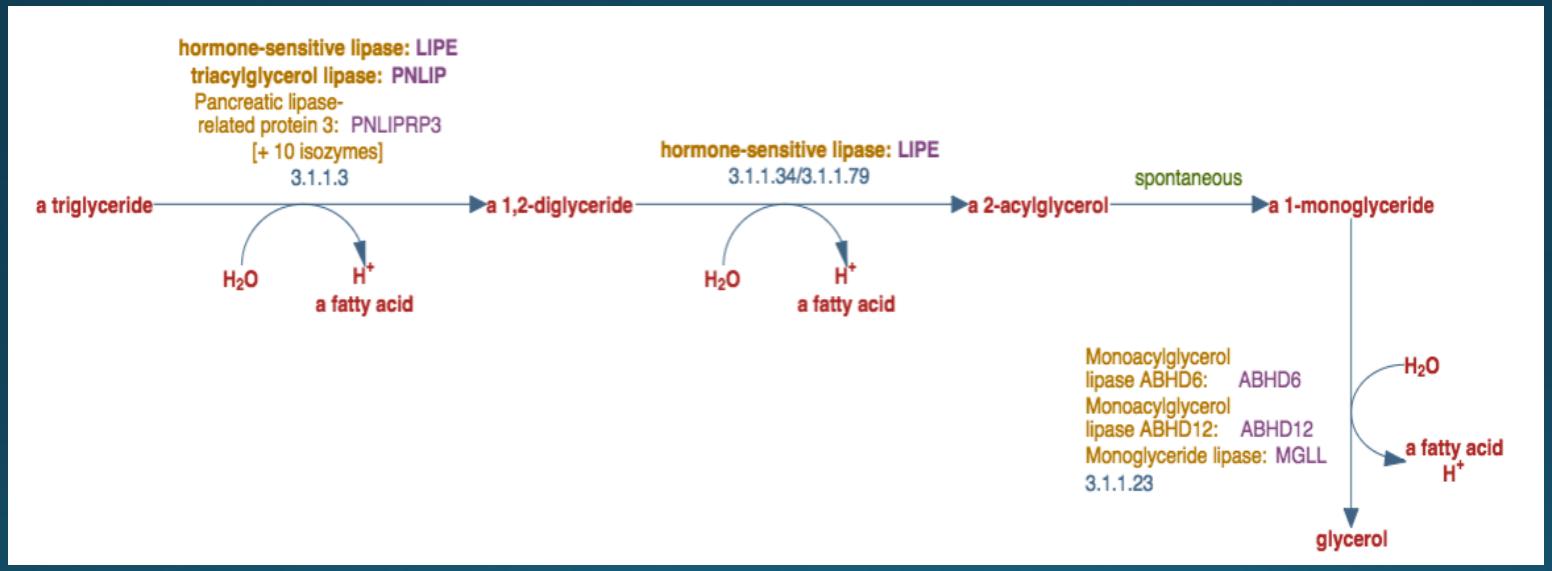




The process produces protons as long as CTP is available

Very interestingly, cancer has long been known to over-produce CTPs

# Increased Triglyceride Degradation

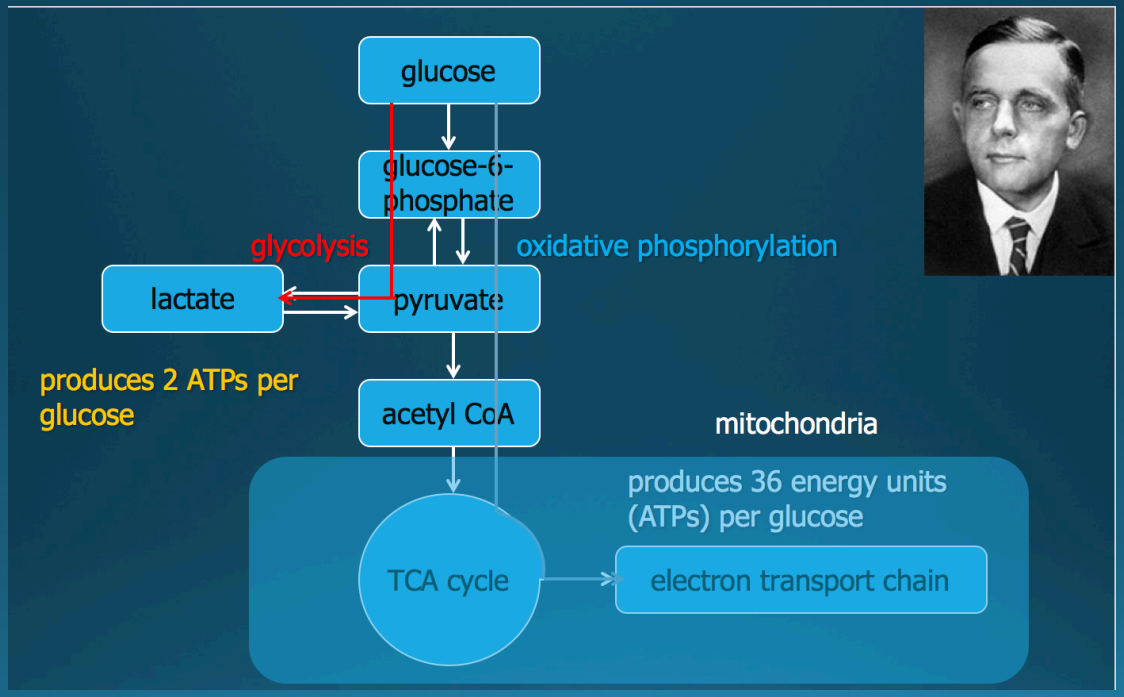
- Cancer also increases the activity of triglyceride degradation




acidification

# Warburg Effect

- This ATP is different from that ATP: each ATP produced by Warburg effect produces one H+ when the ATP is hydrolyzed while ATP produced by respiration is pH neutral!



- ATP generation by respiration
- $ADP^{3-} + HPO_4^{2-} \rightarrow ATP^{4-} + OH^-$

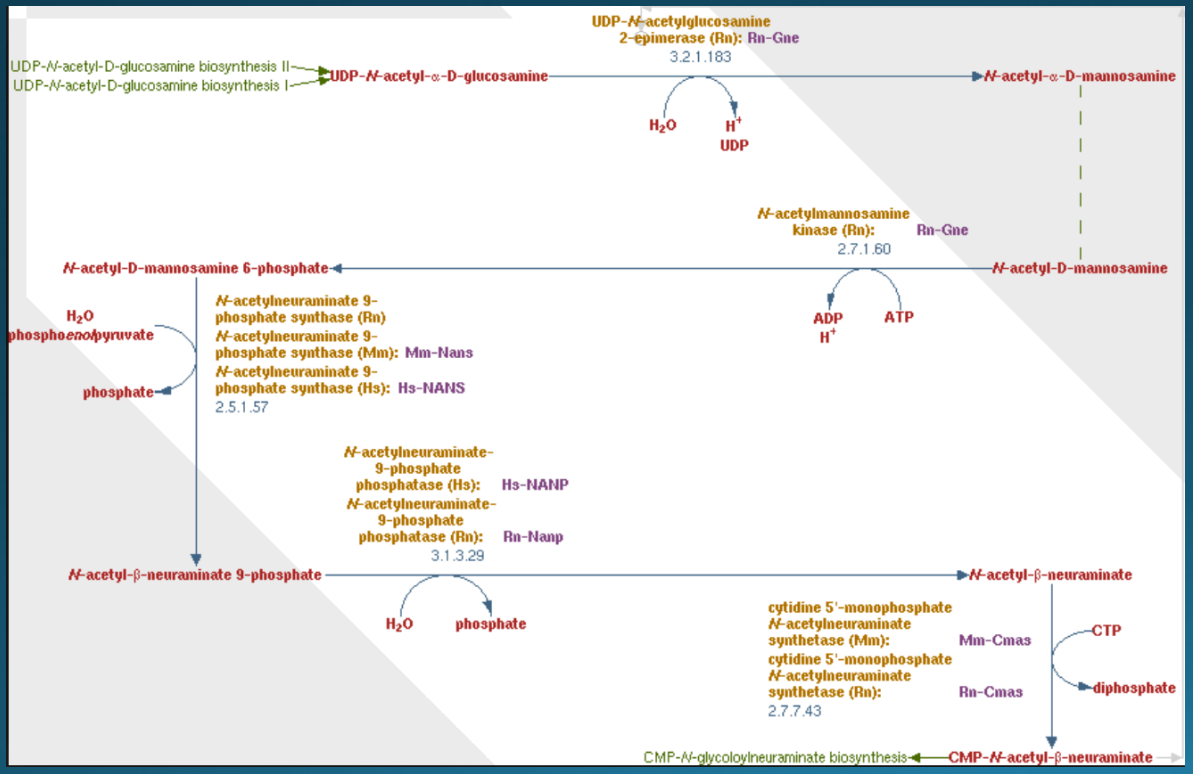
- ATP generation by glycolysis
- $glucose + 2ADP^{3-} + 2HPO_4^{2-} \rightarrow 2\ lactate + 2\ ATP^{4-}$

- Hydrolysis of ATP
- $ATP^{4-} + H_2O \rightarrow ADP^{3-} + HPO_4^{2-} + H^+$

Warburg effect produces more protons than respiration

# Increased Sialic Acid Production

- All cancers tend to gradually increase their sialic acid synthesis as the disease advances



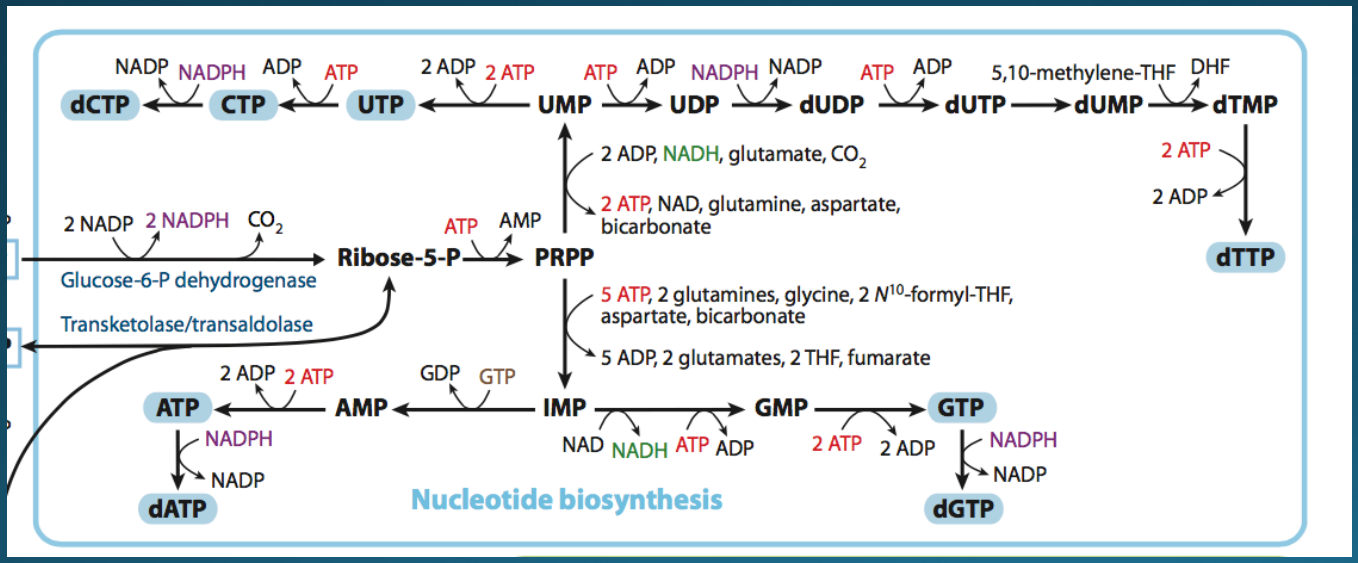

Sialic acid synthesis

acidification

# Nucleotide Metabolic Reprogramming

- Cancer tends to *de novo* synthesize nucleotides rather than uptake via salvage

- Cancer generally synthesizes considerably more purine and pyrimidine

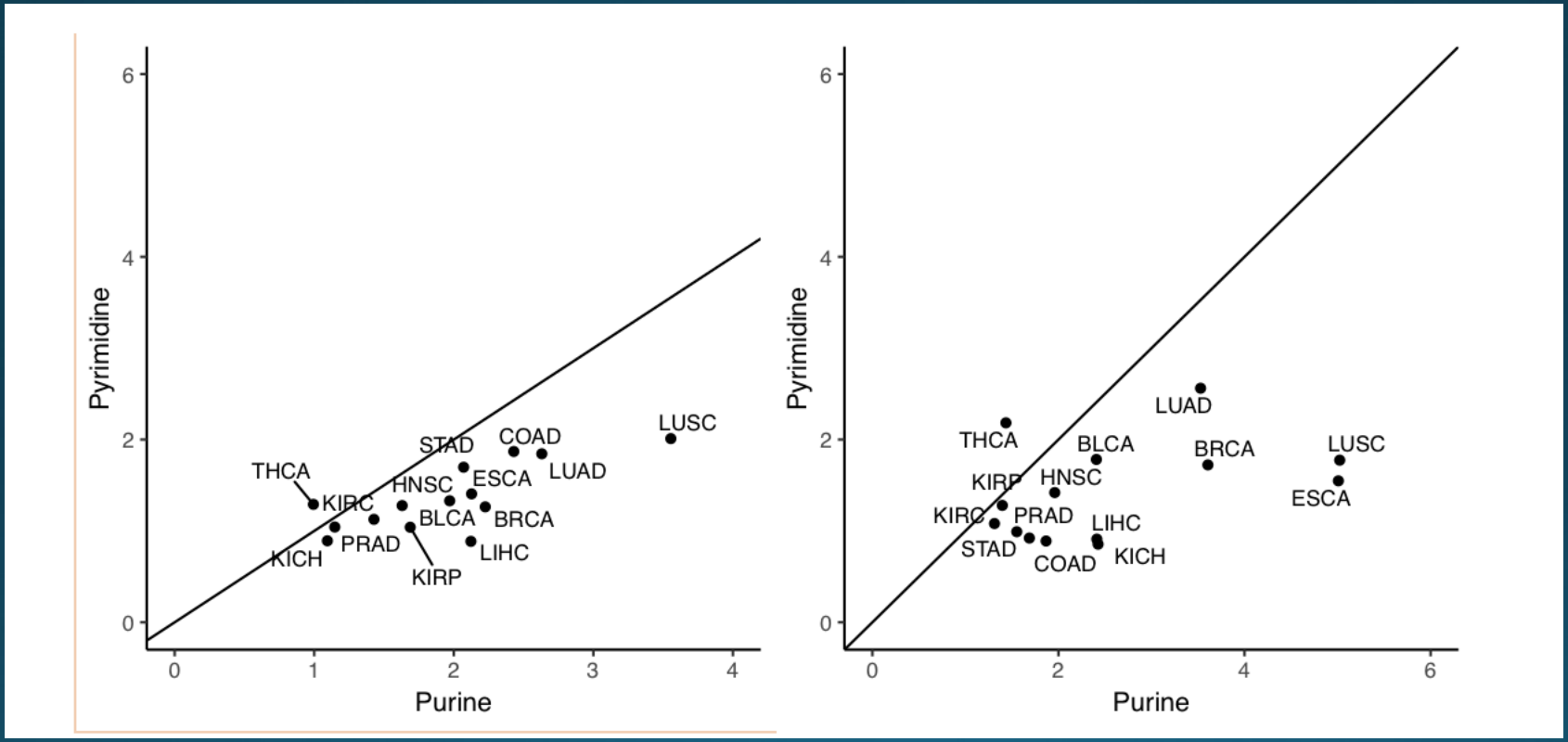



purine

pyrimidine

Purine synthesis consumes more ATPs than that of pyrimidine

# Nucleotide Metabolic Reprogramming




Levels of up-regulation of purine vs. pyrimidine synthesis

## Pyrimidine *de novo* synthesis:

5 ATP + 2 $H_2O$ + 2 glutamine + aspartate + 5-phospho-D-ribose-diphosphate -> dCDP + $H_2CO_3$ + 5 $H^+$;

5 ATP + $H_2O$ + glutamine + aspartate + 5-phospho-D-ribose-diphosphate -> dTDP + $H_2CO_3$ + 3 $H^+$.

## Pyrimidine salvage pathway:

2 ATP + $H_2O$ + 2-deoxycytidine -> dTDP;

2 ATP + 2-deoxycytidine -> dCDP + $H^+$;

2 ATP + thymidine -> dTDP + $H^+$.

## Purine *de novo* synthesis:

5 ATP + GTP + $H_2CO_3$ + glycine + 2 aspartate + 5-phospho-D-ribose-diphosphate -> dADP + 8 $H^+$;

5 ATP + $NAD^+$ + $H_2CO_3$ + $H_2O$ + glycine + aspartate + glutamine + 5-phospho-D-ribose-diphosphate -> dGDP + NADH + 9 $H^+$.

## Purine salvage pathway I:

ATP + 2-deoxyadenosine -> dAMP + $H^+$;

ATP + 2-deoxyguanosine -> dGMP + $H^+$;

## Purine salvage pathway II:

2 ATP + adenosine -> dADP + $H_2O$ + $H^+$;

ATP + GTP + adenosine -> dADP + $H_2O$ + $H^+$;

2 ATP + $NAD^+$ + 2 $H_2O$ + guanosine -> dGDP + NADH + 2 $H^+$;

ATP + guanosine -> dGDP + $H_2O$.

# RNA Pyrimidine and Purine Degradation

- Pyrimidine degradation is known to be persistently repressed or inhibited while purine degradation is considerably up-regulated across numerous cancer types

- The process for pyrimidine degradation is pH neutral and that for purine produces 2 or 3 protons

- Up-/down-regulation is consistent with production/consumption of protons.

# Extensive Metabolic Reprogramming in Cancer

- We have analyzed ~50 reprogrammed metabolisms across 14 cancer types, 7,000+ samples in TCGA

- Found that every reprogrammed metabolism examined produces more protons than the original metabolisms.

# Summary 1

- Cancer tissue cells generally repress proton-extruding transporters and up-regulate proton-absorbing transporters
- A large number of metabolisms are reprogrammed to increase their proton production and decrease proton consumption
- And yet, cancer intracellular pH goes up!
- Why?

# Searching for $OH^-$ Producing Processes

- A long search led us to focus on iron metabolism as it has been long been known that ALL cancers have iron overload

- The reason is that all cancer sites have elevated H2O2 levels, largely due to increased population and activities of macrophages; and red blood cells, which carry iron and O2, tend to oxidized and die here, leading to the accumulation of iron

- The combination of iron overload and increased H2O2 concentration leads to Fenton reaction

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$$

# Persistent Fenton Reactions

- If there are reducing molecules around the reaction, which can reduce $Fe^{3+}$ to $Fe^{2+}$, the reaction will continue

- Our analyses predict that cancer cells use $O2^{-}$ as the main reducing molecule, which is largely from neutrophils

$$O_2^- + H_2O_2 \text{ ---> } OH^- + \cdot OH$$

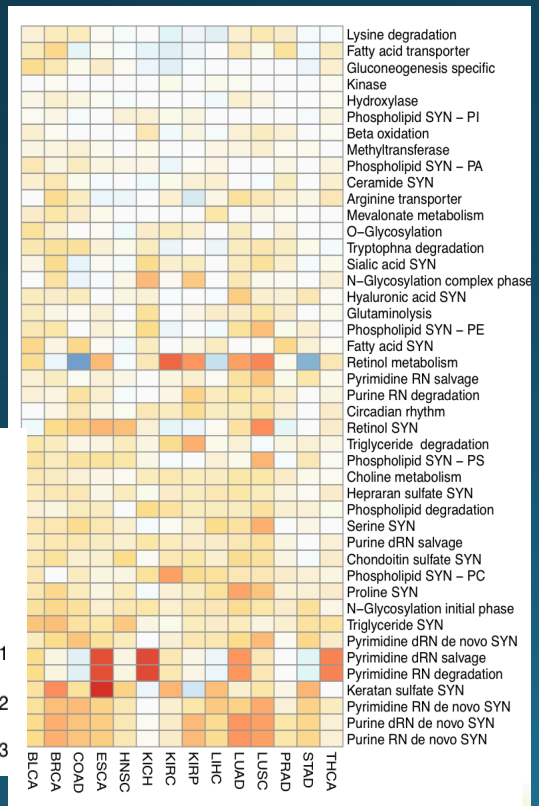
- with $Fe^{2+}$ as catalyst with plentiful superoxide available

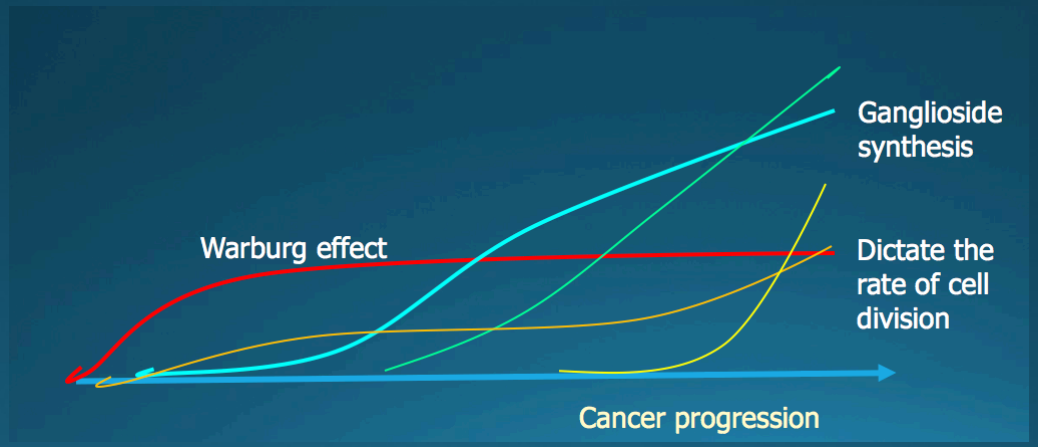
- We predict: all cancer cells have Fenton reactions in their cytosol, and mitochondria; and they overwhelm the pH buffer quickly, hence creating alkaline stress!

Sun et al, JMCB, 2018.

# Metabolic Reprogramming vs. Fenton Reaction

- A regression analysis shows that Fenton reaction level can be well explained statistically by the combination of all the reprogrammed metabolisms in each of 14 cancer types




Based on this and additional supporting evidence, we predict that all the reprogrammed metabolisms are induced to neutralize OH- by Fenton reactions

# Reprogrammed Metabolisms

- Unlike normal metabolisms, reprogrammed metabolisms are triggered to increase proton production but how do the host cells deal with the other products like X and Y?

$$A + B \rightarrow X + Y + H^+$$

- Some are released from the cells such as various hydroxyl-compounds like hydroxyl-proline.

- By for many, finding exits for all such X and Y becomes a new stress for all so affected cells

# Reprogrammed Metabolisms

- Many others cannot be handled this way for two main reasons:
  - some of them are acidic, and hence releasing them will make the cells more alkaline, which cancer generally avoids;
  - some are electrically charged, hence they have to be co-exported with some oppositely charged molecules to maintain electric neutrality; therefore not sustainable.

- For some, the affected cells degrade them, followed by the above producing step, forming a production-degradation cycle, including production and degradation of triglycerides, fatty acids, phospholipids as each such cycle produces net protons!

# Nucleotide De Novo Syntheses

- Nucleotide *de novo* synthesis represents probably the most effective acidifier as synthesis of each purine produces 8-9 protons and each pyrimidine 3-5 protons

- But they cannot be easily exported since they are all negatively charged!

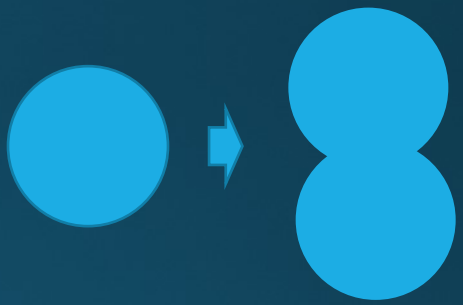
- If they are not released, nucleotide accumulation will slow down and ultimately stop this most powerful acidifier.

# Cell Division Model

The levels of Fenton reactions dictate cell division rates

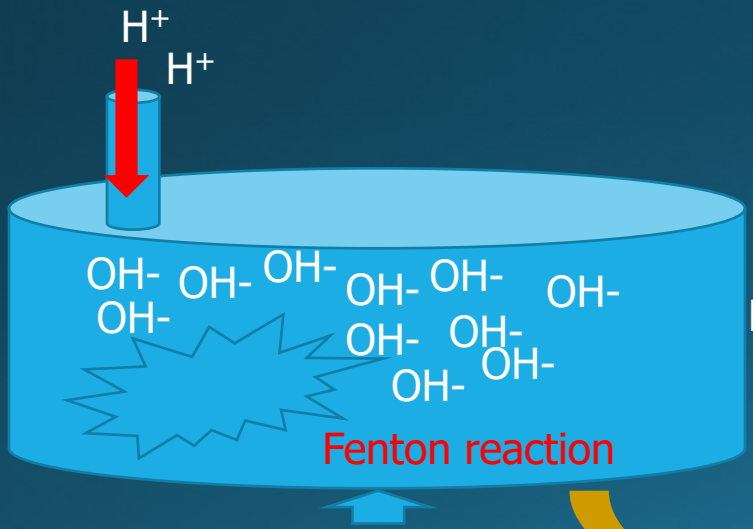
Many, possibly all unicellular organisms use nucleotide-sugar concentration to drive cell cycle

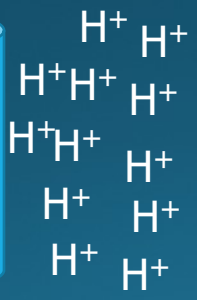
Cell division to rid of nucleotides?



Nucleotide synthesis is a major workhorse acidifier

To sustain, it requires nucleotides be rapidly removed




A range of reprogrammed metabolisms

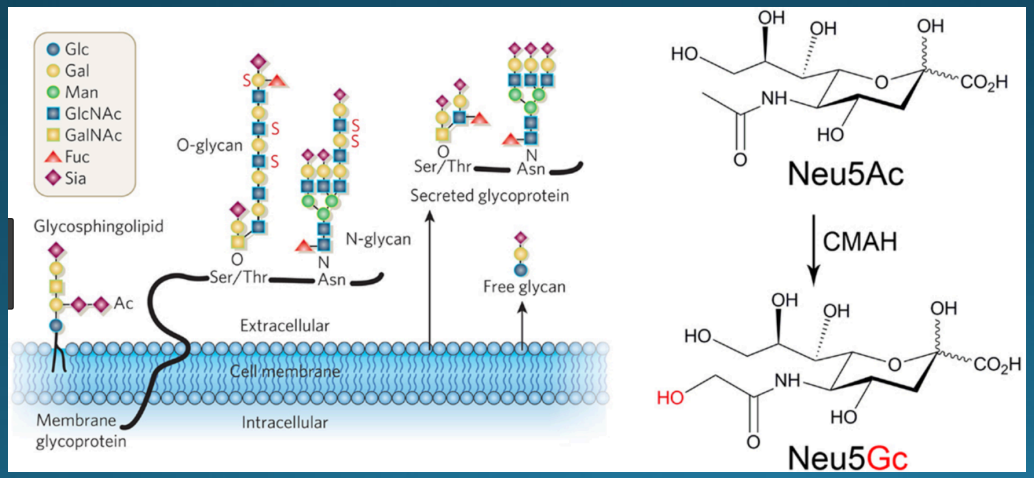
- Warburg effect
- Nucleotide synthesis
- Sialic acids
- .....

Chronic inflammation
Iron accumulation

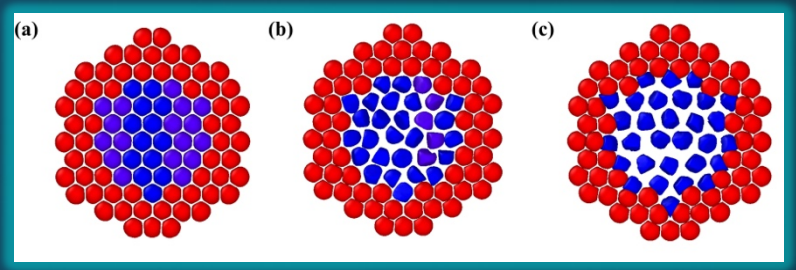
# Sialic Acid Synthesis

- The synthesis of a sialic acid produces two protons and its deployment also produces additional protons (via synthesis of gangliosides)

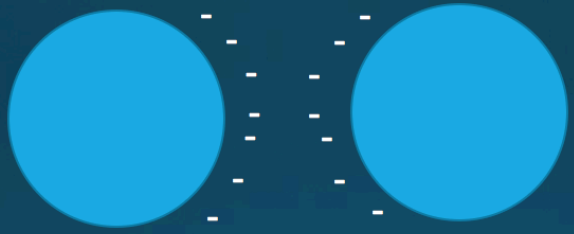
- Sialic acids are generally deployed on cell surface and they are negatively charged



# Metastasis model



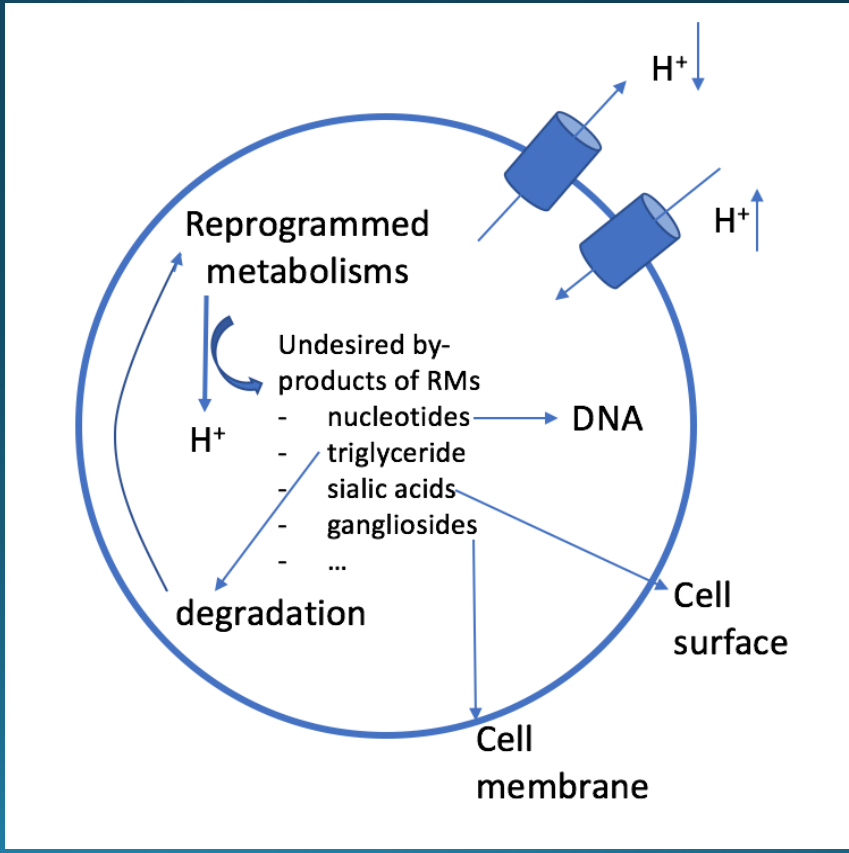

- It is interesting to note that a sialic acid is negatively charged!



- Consequence: The repulsion among the negatively charged cell surfaces alters the shape of the cells, hence activating mechano-sensors such as SNAIL, and drives cells apart, enhancing the cell-cell adhesion, ultimately activating the EMT mechanism for cancer cell migration

# Other Phenotypes of Cancer

- Drug resistance
- Persistent loss of sodium in blood
- Cachexia
- …



# Summary 2

- Metabolic reprogramming is induced to neutralize OH- persistently produced due to chronic inflammation and iron overload

- Finding metabolic exits for some of the reprogrammed metabolisms give rise to a variety of phenotypic behaviors of cancer cells.

# Purposes Served by Mutations

- To ensure that cells can divide at rates comparable with the rates of Fenton reactions
- To prevent execution of various constraints that inhibit cell division illegally
- In general, to enable metabolic reprogramming, hence to enable cell survival

# Take-Home Message

- Chronic inflammation of certain types may play a key driving role of Fenton reactions in multiple subcellular locations

- Persistent Fenton reactions in cytosol and mitochondria may play the key driving roles in cell level metabolic reprogramming

- Clinical behaviors of cancer, including cell division metastasis, drug resistance, …, cachexia, may be the results of these reprogrammed metabolisms, either to provide metabolic exits in a sustained manner or to maintain fundamental properties of cells such as electric neutrality

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