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Symposium website: www.cancerevolution.org

Evolution of Evolutionary Processes and of Evolvability as a Trait in Organismal and Cancer Evolution

Real-time genome and molecular phenome evolution of cancer unicellular quasi-species
Real-time co-evolution/development of host immune system, ECM and TME
Increasing cancer cell glycosylation and fast, 'heritable' glycome evolution during progression

October 16, 2020 – 12:15 pm ETD

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Evolution of Evolutionary Processes in Organismal and Cancer Evolution

Nature does not differentiate between normal biology and pathobiology. These artificial distinctions obscure how flexibly and 'agnostically' cell biology will employ the most efficient ('fittest') molecular mechanisms, regardless of whether they are adaptive in organismal evolution, or whether they promote infections or cancer within a host.

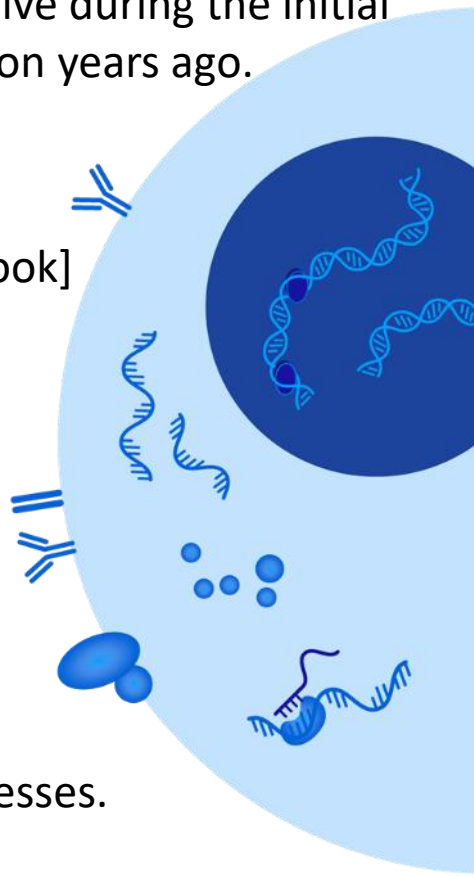
- Via on-going evolution of **efficient evolutionary processes**, nature leverages any **evolvable** biological information storage and processing mechanism, as well as any signaling or interaction process for new pathways and mechanisms of action.
- In organismal evolution, billions of years of adaptive evolution of modern cell and organismal biology have facilitated more flexible, faster and more likely to succeed natural genetic engineering (NGE) and epigenetic/phenomic active ***change*** generation, prior to Darwinian ***selection***.

➔ **Organisms and cancer cells both evolve towards improved evolvability as a 'trait'** [also see Ken Pienta publication]

- This compares to inefficient, infinitesimal and random DNA mutations, which by now mostly have a neutral or deleterious effect in organismal evolution, and today likely have a negligible impact on adaptive organismal evolution.
- The prevailing random mutations theory of organismal evolution has been made mostly obsolete by Nature in contemporary cell biology, after 3.5B-4B years of evolution of efficient, feedback-driven or vector-mediated processes.
- The remaining, very visible exceptions are cases where random mutations have deleterious effects, e.g. by causing deformations, death, *monogenic* diseases, or by triggering **driver mutations in oncogenes or tumor suppression genes**.
- More efficient, active and 'fitter' evolutionary processes have dramatically accelerated organismal evolution, in a manner that now can be reconciled with the ***punctuated fossil record***, and the accumulating ***DNA record*** of organismal evolution.

Evolution of Evolutionary Processes and Evolvability in Unicellular and Multicellular Organismal Evolution

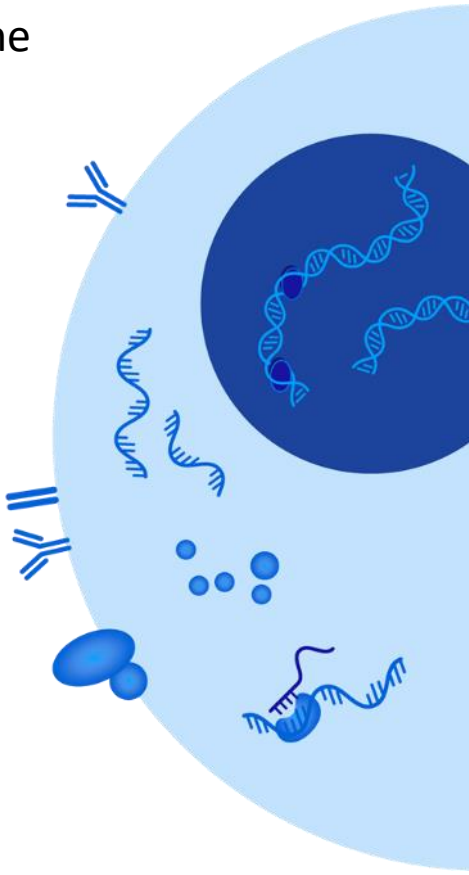
- An important recognition is that active, evolutionary cell biology processes first had to evolve during the initial ~2 billion years of single-cell evolution, after the origin(s) of life on Earth some 3.5-4.0 billion years ago.
- Subsequently, in more recent 1-2 billion years of life on Earth, these evolved, active change generation processes have greatly accelerated adaptive evolution.
  - Various ***natural genetic engineering (NGE)*** cell biology processes [see J. Shapiro reviews and book]
  - Complemented by horizontal gene transfer (HGT), symbiogenesis and viral transduction
  - [Bonner et al.: EVs facilitate 'HGT' between cancer cells and other host cells in metastasis]
- This has enabled the emergence of **eukaryotic** single-cell life and culminated in the multiple origins and rapid evolution of complex **multicellular life**.
- After some 3.5-4 billion years of life and biological evolution on Earth, not only of traits and species, but importantly also of **evolutionary processes** themselves, nature's evolved feedback processes today can **actively modify, restructure, rewrite and rearrange genes, chromosomes, 3D genomes and karyotypes**, via numerous intra-cellular, intra-organismal (e.g. EVs) and vector-transmitted processes.
- In Darwinian manner, these often major and sometimes already directionally functional changes can be selected for by nature according to their **differential phenome fitness**.



➔ As selected, adaptive changes accumulate over time they also evolve **more efficient evolutionary processes and faster evolvability,** in addition to advantageous traits and to new species with improved fitness.

Evolution of Evolutionary Processes and Evolvability

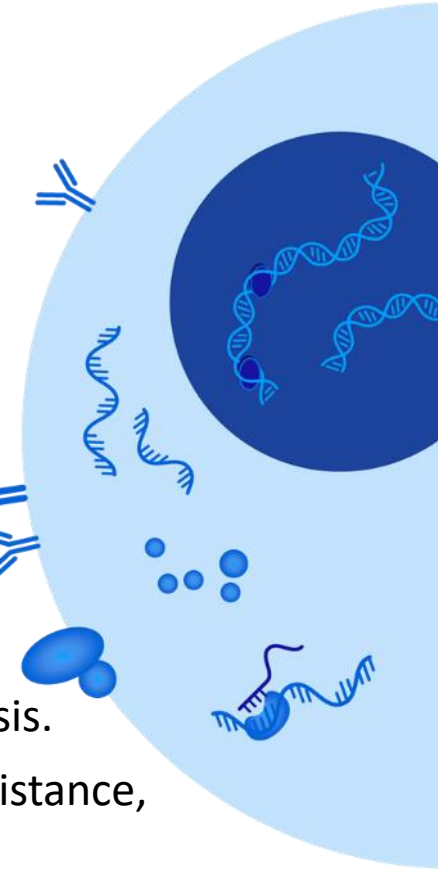
Adapted from '***Evolution of Evolvability***' by Guenter P. Wagner and Jeremy Draghi, chapter 15, '*Evolution: The Extended Synthesis*', book edited by M. Pigliucci, G.B. Mueller, MIT Press, 2010.



- Wagner & Draghi explained why the arguments ['hostility of some population geneticists'] *against* the **evolution of evolvability by Darwinian selection** favoring more evolvable genotypes are *incorrect:*
  - Evolution of evolvability has been a 'self-inflicted blind spot' in the Modern Synthesis theory, e.g. due to its *philosophical* insistence on random mutations as only 'permitted' variation prior to selection
  - Multiple ways to assess **short- and medium-term evolvability** with artificial selection data using population genetic principles ➔ **evolvability selected due to higher mean fitness over time**
  - **More evolvable haplotypes, a distributed, omnigenic property of the genome, increase in frequency**
  - Philosophical argument against appearance of teleology as selection cannot predict the future: there is no paradox, as recurrent environmental threats and opportunities select **more evolvable survivors**
  - Argument that sexual recombination prevents selection of evolvability is erroneous (as for all traits)
  - ***Survival of the most evolvable* as important as survival of the fittest**
  - Example: link between success of invasive species and evolvability (just like in cancer metastasis)
  - Insight: evolvability-enhancing changes expected to **affect evolvability of surface proteins**
- **Evolvability** is a relational concept that reflects the systemic contributions to evolution by:
  - amount of genetic variation
  - plasticity/variability of the phenotype for a given genotype [see Denis Noble talk 'tissue stochasticity']
  - weak or strong selection, and
  - subject to mechanistic, developmental constraints or canalization in trait space (variability not 'isotropic')

Cancer Evolution of Evolutionary Processes and Evolvability

Real-time multiclonal cancer cell quasi-evolution:



- Both, random ‘driver’ mutations and active, major gene, chromosomal and genome modifications of heterogeneous cancer cells **modify the cancer cell genome, karyotype**
- Evolving cancer cells exhibit frequent cancer WGD, aneuplodies, polyploidy and multinucleated giant cancer cells (Pienta’s PACCs, also Liu), which are quiescent and therapy resistant
- Together with **cancer cell epigenome and transcriptome changes**, cancer evolutionary processes also change **cancer cell phenotypes**, expressed as proteins, peptide neoantigens, glycosylation, other PTMs, lipids, metabolites, as well as EVs and the exosome.
- **Cancer evolutionary processes** do not only change, select and evolve cancer cells themselves, also **shape the tumor microenvironment in ‘niche construction’ in cancer** (Adelene Perkins talk)
- Real-time **co-evolution/co-development of the host immune systems**, i.e. of the patient, plus various forms of therapy can either suppress or facilitate cancer progression (hyper-progression) and metastasis.
- Typically, if cancer cell populations do not become extinct (Gatenby, Audeh), they develop therapy resistance, which is strongly correlated with recurrence, increased adhesion/invasiveness and metastasis.
- Opportunity to track cancer longitudinally with **biomarkers of cancer evolution** (Anne Barker talk)

➔ Early detection and biomarkers of cancer evolution **most sensitive and with highest PPV if cancer cell (epi-) genotype, karytope and phenotype are detected**, **AND if host/immune/TME response** to cancer cell evolution is measured

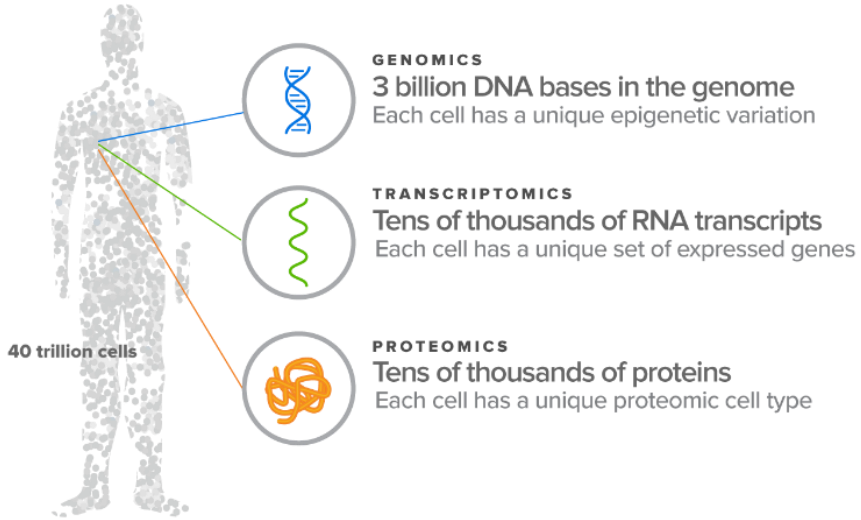
Implications of Cancer Evolution for Early Detection with Higher Sensitivity, Excellent Specificity, Improved PPV and useful TOO localization

'First cancer cell' Early Detection vs. MRD, Therapy and Recurrence Monitoring

- The March 2020 Liu et al. Annals of Oncology publication 'Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA' (doi.org/10.1016/j.annonc.2020.02.011) shows the way for **early pan-cancer detection with excellent specificity and much improved positive predictive value (PPV), plus remarkable tissue of origin (TOO) localization** to facilitate subsequent imaging (e.g. LD-CT, PET)
- However, there is still a lot of room and a real medical need for further early-stage sensitivity and PPV improvements
- CF-DNA methylation analysis needs to be complemented by higher precancerous and early cancer stage I and II sensitivity for single or targeted few or single cancer early detection and stratification, e.g. by cfDNA or cfRNA genome using NGS
- **Complement cancer-cell derived cell-free DNA, methylation and RNA signatures** with measurements of **non-cancer cell derived host response biomarkers**.
- Indirect detection adds monitoring the **patient as 'inherently most sensitive detector' of early cancer**. This will happen at the cfNA level, e.g. to detect **host immune** responses to precancerous or stage I and II stimulation of host immune system.
- Even more sensitive early cancer detection will also benefit from liquid biopsy measurements and machine learning of characteristic protein, PTM, PPI, and metabolic/lipidomic profiles.
  - For complementary **host response signals**, expect **inherently lower specificity** than for cancer-cell derived signatures
  - But **early stage sensitivity is bound to be high, for improved early stage PPVs** with moderate-good specificity
- Combining multiomics liquid biopsy approaches may be the breakthrough in **high PPV early detection of cancers**.
- **Important caveat**: cancer evolution suggests that early detection markers differ significantly from MRD or recurrence biomarkers

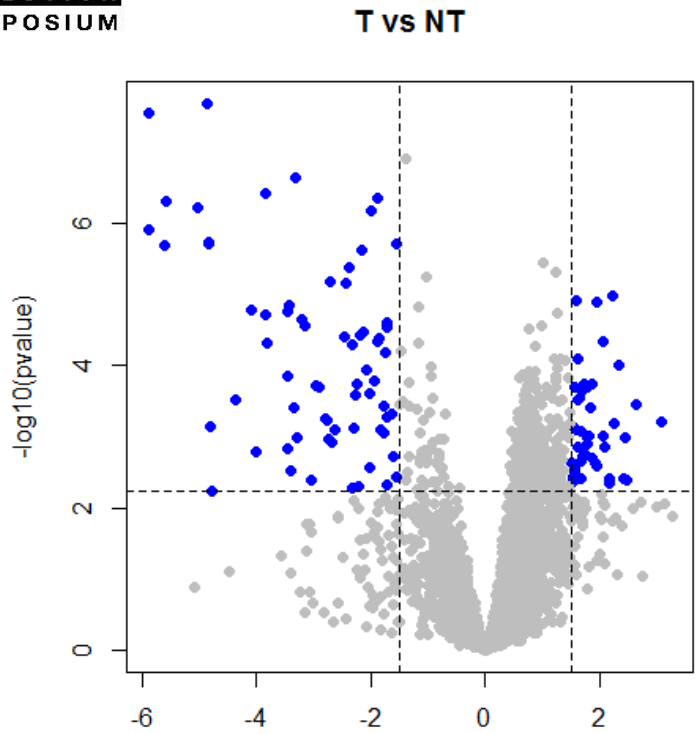
Evolution of Cancer Cell Genome and Phenotype
Co-evolution/development of host immune system and TME

- Gradual and punctuated **cancer cell** evolution work at the **genetic, epigenetic and 3D genome level**:
  - somatic mutation: founder, driver and passenger mutations in proto-oncogenes (Ras, EGFR, HER2) and tumor suppressors (p53)
  - feedback-driven, active DNA insertions and rewrites via reverse transcription, viral or exosome vectors or other NGE processes for major, non-stochastic alterations in genes and DNA regulatory sites
  - 'Heritable epigenetics' of unicellular cancer quasi-species, analogous to tissue differentiation in development (e.g. methylation)
  - Genome destabilization (aka genome 'chaos') and large-scale genome reorganizations, e.g. via *kataegis* (localized hypermutation) or *chromothryspis* (clustered chromosomal rearrangements)
  - 3D genome changes, aneuploidies, polyploidy and multinucleated cells
- Explore **cancer cell** evolution of **gene expression and phenotype inheritance**:
  - Quantitative **transcriptomics**, fusion RNA genes, the 'living genome'
  - Quantitative **proteomics, post-translational modifications (PTMs)** of cancer cells
  - **Peptidomics** and novel 'heritable' **surface neoantigens**
  - Apparently, '**heritable' functional glycosylation** and shield against immune system
  - **Extracellular vesicles (EVs) and exosome** role in adhesion, invasiveness and metastasis
- Explore real-time evolution/development of **host response** and **cancer modulation/suppression of immune response**



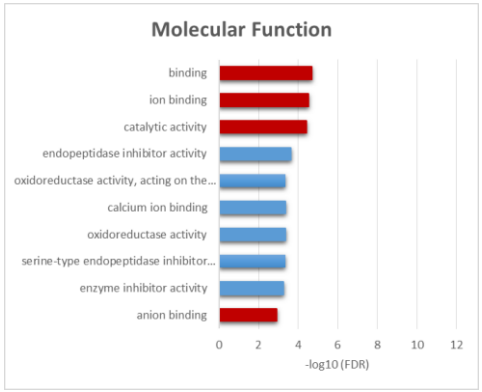
How Does the Cancer Cell Proteome Evolve? Example: Mouse

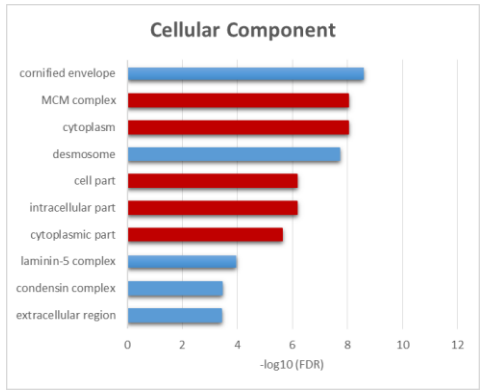
Gastric Cancer Proteins Differentially Expressed in Tumor vs. Non-Tumor Cells

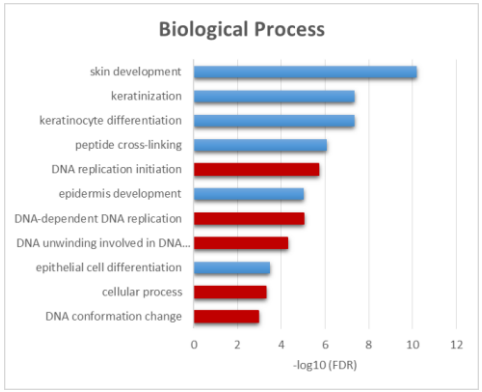


Volcano plot of 5,000 identified proteins comparing tumor and non-tumor tissues.

Gene Ontology (GO) enrichment analysis of 110 significant tumor-associated proteins:







The mini-chromosome maintenance protein **complex** (**MCM**) is a DNA helicase essential for genomic **DNA replication**. The MCM complex is linked to **genomic instability** and a variety of carcinomas. MCMs shown to promote cell proliferation in certain cancers by enhancing DNA replication. High expression of MCM7 (Minichromosome Maintenance Complex Component 7) is correlated to gestational choriocarcinomas (in uterus), lung cancer, papillary urothelial neoplasia, esophageal cancer, prostatic intraepithelial neoplasia, and endometrial cancer.

Can cancer cell protein expression be predicted from DNA or RNA?

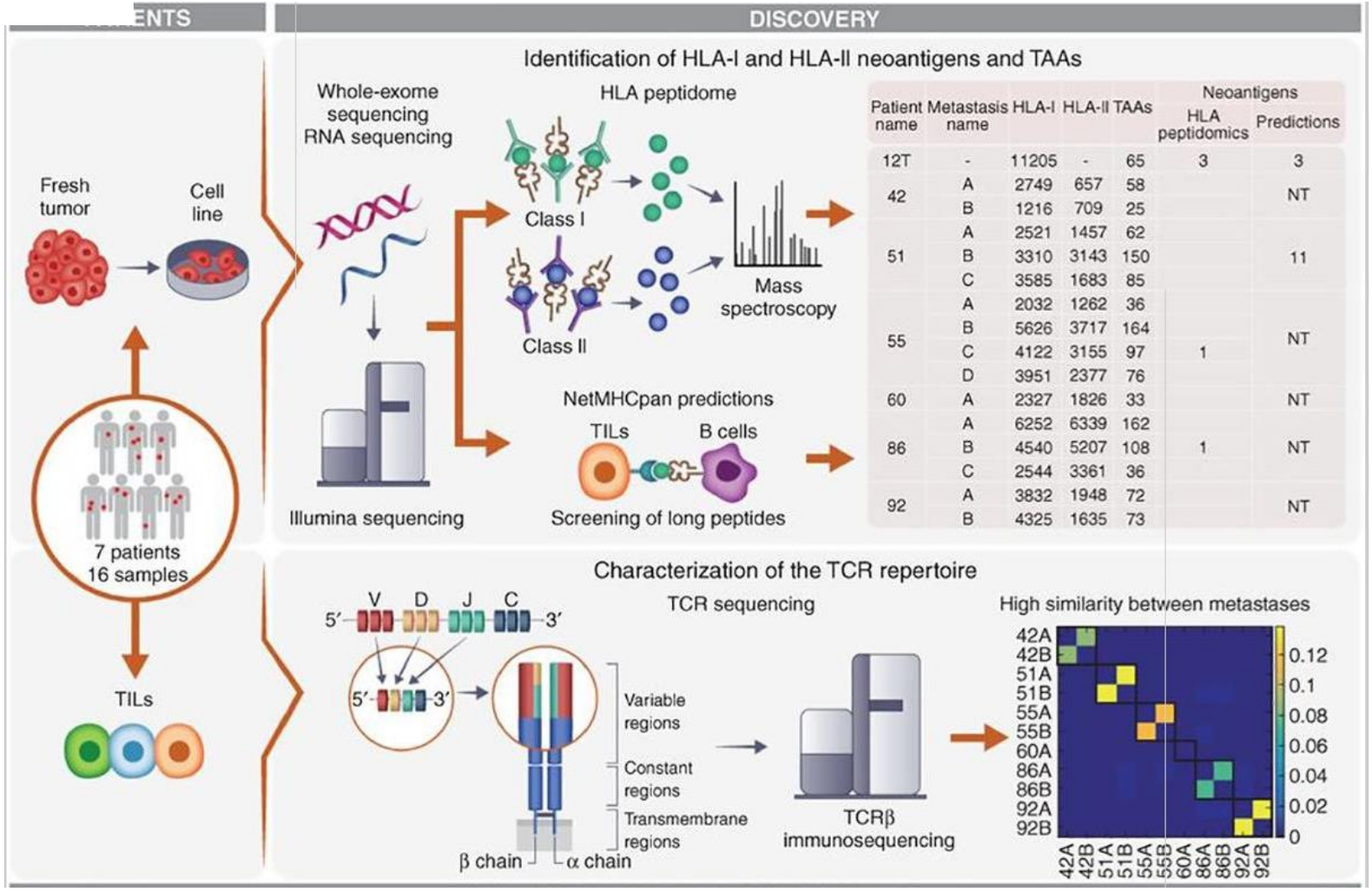
➔ **No, correlations between mRNA and protein expression are poor, sometimes uncorrelated or anti-correlated**

Is all information about cancer cell protein expression stored in some genetic or DNA 'blueprint'?

➔ **Blueprint metaphor is wrong. Genes are a parts list, with distributed heritable and evolvable biological information**

Evolution of Cancer Cell Surface Peptides:

Neoantigen Peptides Evolve from Human Leukocyte Antigen (HLA) Proteins Encoded by Major Histocompatibility gene Complex (MHC)



Patient name	Metastasis name	HLA-I	HLA-II	TAAs	Neoantigens HLA peptidomics	Neoantigens Predictions
12T	-	11205	-	65	3	3
42	A	2749	657	58		NT
	B	1216	709	25		
51	A	2521	1457	62		11
	B	3310	3143	150		
	C	3585	1683	85		
55	A	2032	1262	36		NT
	B	5626	3717	164		
	C	4122	3155	97	1	
	D	3951	2377	76		
60	A	2327	1826	33		NT
86	A	6252	6339	162		NT
	B	4540	5207	108	1	
	C	2544	3361	36		
92	A	3832	1948	72		NT
	B	4325	1635	73		

Requirements for Discovery:

- NGS
- High sensitivity peptidomics MS/MS sequencing
- Software for HLA peptidomics

Figure 1 from: Kalaora et al., Cancer Discov. 2018 Nov;8(11):1366-1375. doi: 10.1158/2159-8290.CD-17-1418.

T-cell receptor (TCR): generated through random rearrangement of genomic V(D)J—variable (diversity) joining—segments. TCR is the mediator of specific antigen recognition by T lymphocytes.

Neoantigens are new antigens not recognized by the immune system. Neoantigens can arise from altered tumor proteins formed as a result of tumor evolution (or from new viral proteins).

➔ Cancer cell 'unicellular' quasi-species evolve their genomes and protein/PTM/peptide phenotypes during real-time cancer evolution

Cancer Peptidomics:

Neoantigen train immune system for enhanced killing of cancer cells by TILs in immune checkpoint therapy (ICT), e.g. CAR-T immunotherapy, or to develop personalized patient cancer vaccines

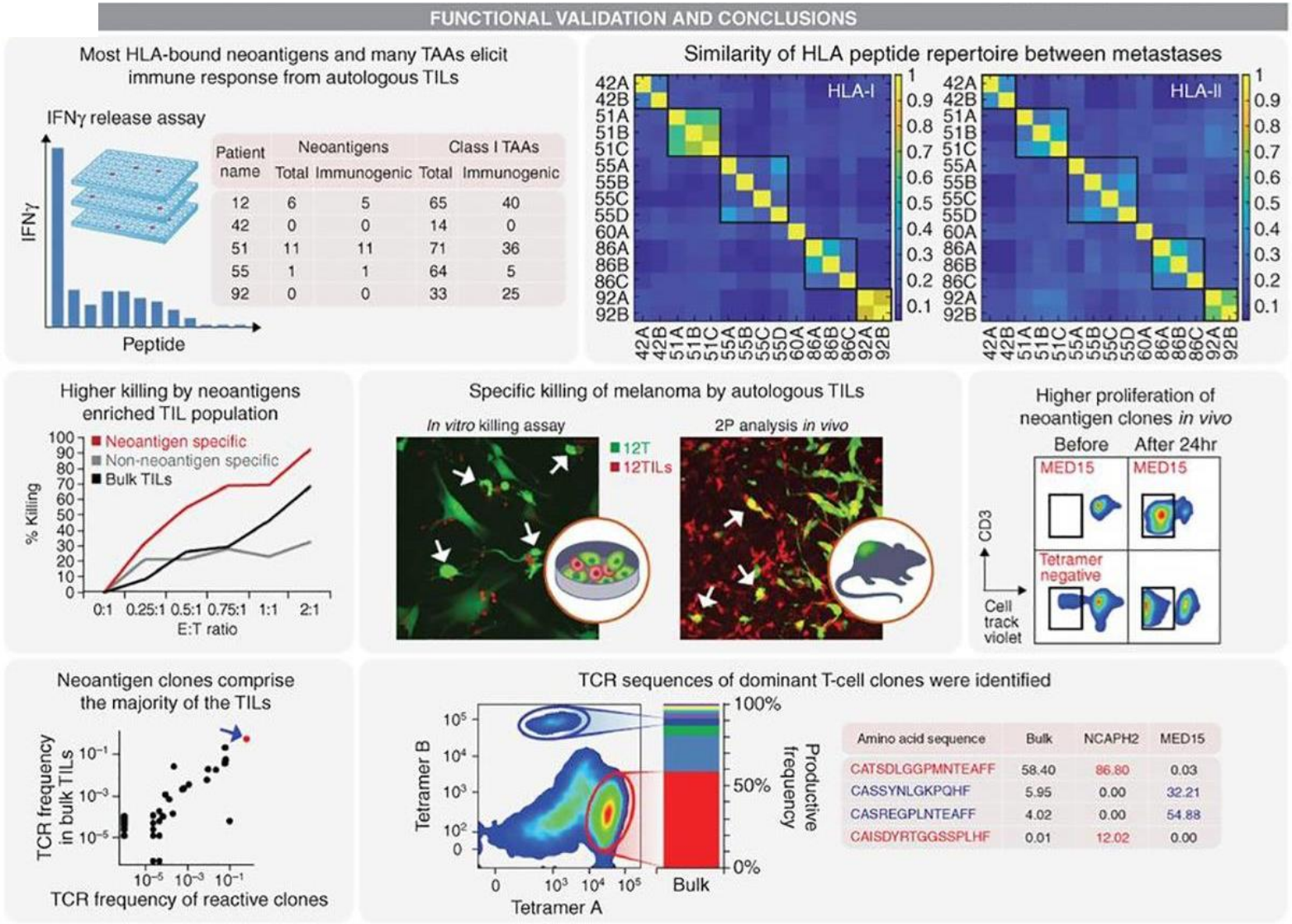


Figure 1 from: Kalaora et al., Cancer Discov 2018 Nov;8(11):1366-1375. doi: 10.1158/2159-8290.CD-17-1418. Epub 2018 Sep 12

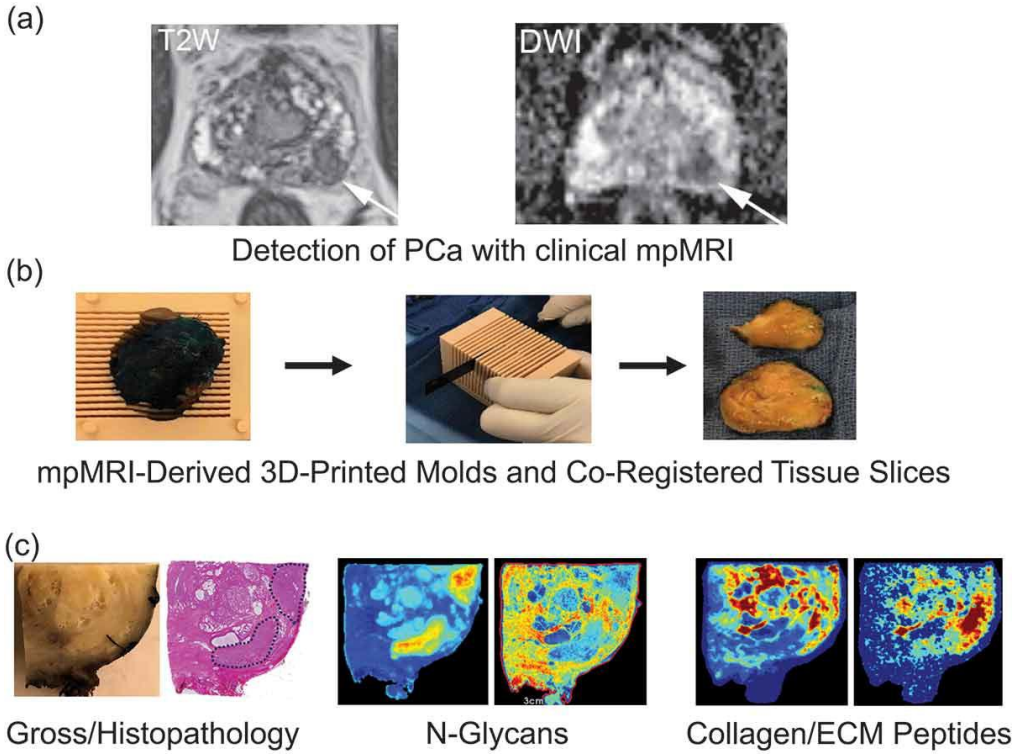
- Tumor infiltrating lymphocytes (TILs)
- Chimeric Antigen Receptor T-cells (CAR-T)

But: multiclonal cancer species continue to evolve during (immuno-) therapy

- **Increasing cancer cell 'decoration' by glycosylation shield evolves to evade immune system**
- Similar to evolving viral protein glyco-sylation shield in HIV, SARS-CoV-2
- Novel glycoproteomics workflows tackle **cancer cell evolution at glycomics level**
- Cancer cell proteomics and glycomics **will find additional cell phenome biomarkers for early detection, or later MRD/therapy monitoring**

Evolution of Cancer Cell Glycosylation, Malignancy of Stroma and ECM:
Glycomic cancer cell surface and ECM biomarkers in Prostate Cancer (PCa)

Figure: **Workflow to evaluate glycomic and extracellular matrix (ECM) biomarker candidates for prostate cancer**



- Compositional and structural variability of glycans on glycoproteins and glycolipids is unsurpassed in Nature.
- Multiple types of complex carbohydrates comprise the cell surface **glycocalyx.** Glycans include N-linked and O-linked glycoproteins, glycosphingolipids, others…
- Cell surface glycans interact with stroma extracellular matrix (ECM) proteins, many of which are also glycosylated
- **Cancer cell surface glycans interact with various cell types, including immune cells**

➔ **Progressively 'decorated' cancer cells use evolving glycan shield against immune recognition**

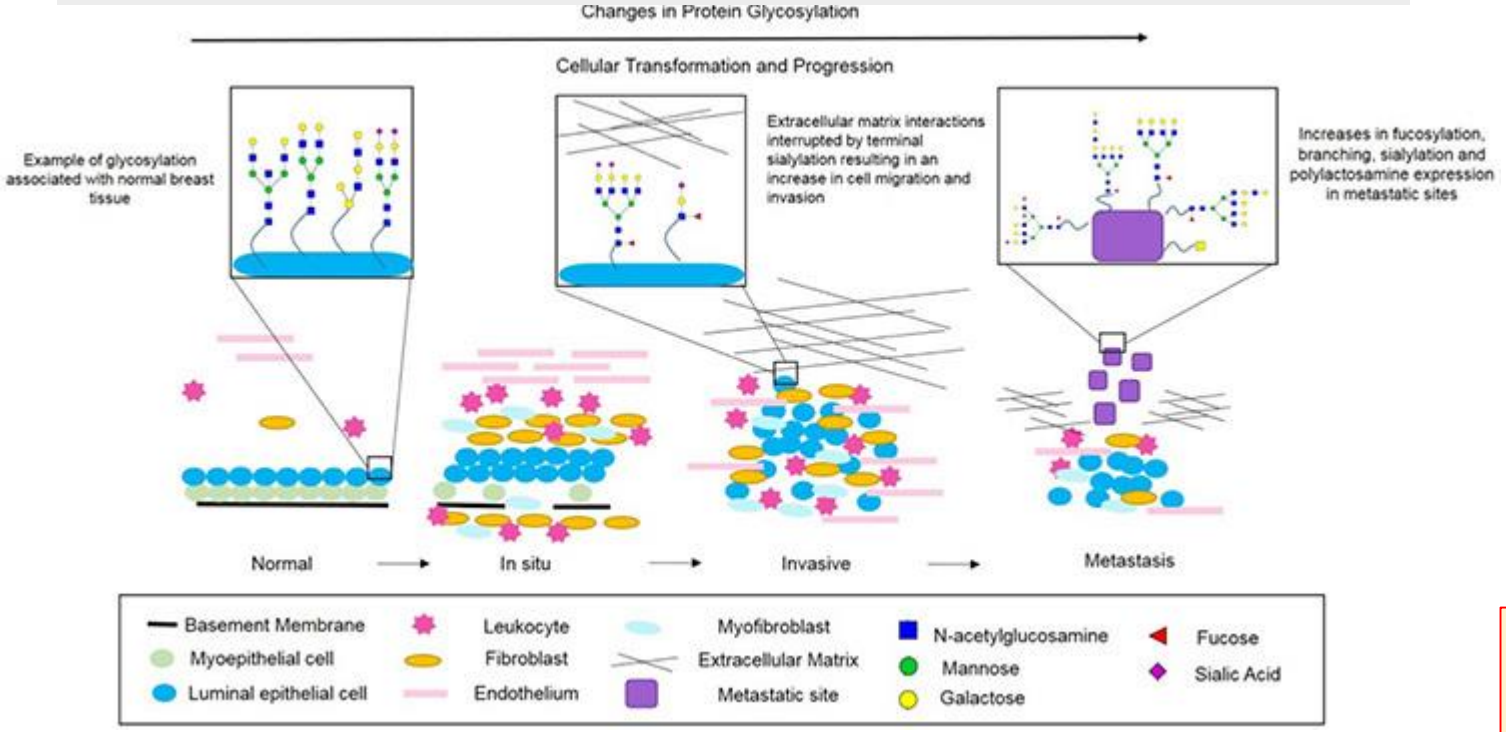
- Role of **stroma in promoting cancer progression, e.g. PCa is complex: malignant transformation of ECM** disrupts homeostatic microenvironment, **altering tissue processes of adhesion, as well as cancer cell death, migration, and proliferation in tumor growth**

Richard R. Drake, Peggi M. Angel, Jennifer Wu, Russell K. Pachynski & Joseph E. Ippolito (2020) How else can we approach prostate cancer biomarker discovery?, Expert Review of Molecular Diagnostics, 20:2, 123-125, DOI: 10.1080/14737159.2019.1665507

Evolution of Cell Glycosylation during Cancer Progression

Changes in N-linked and O-linked Glycans in Breast Cancer Tumors and Biofluids

Figure: **As a cancer becomes invasive and metastatic, N-glycans expressed display increases in branching, sialylation and fucosylation. O-glycans decrease in chain length and expression of Tn antigen, Thomsen-Friedenreich antigen, sialyl-T antigen and sialyl-Tn antigen increases.**



Scott DA, Drake RR. Glycosylation and its implications in breast cancer. Expert Rev Proteomics. 2019 Aug;16(8):665-680. doi: 10.1080/14789450.2019.1645604.

- Changes in glycosylation at tumorigenesis
- Alterations in glycosylation functions implicated in metastasis
- Attempts at deciphering glycosylation changes associated with breast cancer progression
- Glycomics analysis lags behind genomics and proteomics, but new approaches emerging (e.g. PNGase F in mass spec imaging)

➔ **Dynamics of tumor and immune glycosylation critical to improving immunotherapy**

Separately, cancer metabolism needs more glucose than normal cells for rapid proliferation. We detect tumors using PET imaging of radioactively labeled sugars. Lower carbohydrate diets can slow tumor growth in mice. *doi.org/10.1038/s42003-019-0455-x*

Evolution of Cell Glycosylation during Cancer Progression

How is glycosylation information transmitted and 'inherited' by progeny cancer cell generations in cancer proliferation?

- A substantial fraction of genes code for proteins involved in carbohydrate synthesis, activation and transport as well as glycan assembly, modification, remodeling and degradation.
  - Example: *ABO* gene encodes glycosyltransferase enzyme to modify carbohydrate antigens on red blood cells.
- BUT: glycosylation does not appear to be templated, unlike transcription or translation
- Glycans are major determinants of cellular interactions and contribute to the composition of the ECM.
  - Example, glycans as docking sites for bacteria and viruses, such as glycosylated RBD of SARS-CoV-2 spike protein
- Partial understanding of intricate regulation of expression and functions of glycan epitopes is replacing view as purely phenomenological feature with a random, complex profile, e.g. as implied by earlier term '*glycocalyx decoration*'.

➔ **Concept of evolvable, carbohydrate-based *Sugar Code*, the *Third Alphabet of Life***

- **Sugar code is only partially understood, and partially still an enigma that is still being decoded**
- **It might use 'fuzzy logic', and not always precise algorithms and templated, causal relationships (like DNA code)**
- **From an information storage, processing and signaling perspective, the Sugar Code may have similarities with the hypothesis of the epigenetic (histone and methylation) code**

'***An introduction to the sugar code***', by Hans-Joachim Gabius & Jürgen Roth, *Histochemistry and Cell Biology*, **v. 147**, pages111–117 (2017).

Evolution of Cell Glycosylation during Cancer Progression

Evolvable, Carbohydrate-based *Sugar Code*, the *Third Alphabet of Life*

- Density of information must be very high on cell surfaces, because space for multitude of different signals is limited

➔ A different class of biomolecule is required with **capacity to allow flexible synthesis and frequent branching for many more isomers ('words') from structural glycan subunits** than NAs or AAs permit.

- Monosaccharides are the letters of the sugar code of the third (glycome) alphabet of life, after the NA and AA alphabets
- Sugar code allows **very high-density coding capacity**, and the glycome is very complex with **inherently fast dynamics**
- The sugar code and other PTMs are not templated by the universal DNA code, which predicts mRNA and AA sequence.
- Sugar code is read by **lectins**, the carbohydrate-binding proteins that can be semi-specific for glycosylation 'groups'
  - Specificity of homologous lectin groups is not as high as antibody-antigen, or antisense RNA, or CRISPR specificity
  - But glycoprotein and glycolipid interactions with lectin **exhibit fast dynamics, and group selectivity**
- **Permits a type of faster, higher density, less specific biological information processing and signaling** (akin to cell ML?)
- **Enables fast, transferable and 'heritable' phenotype evolvability and adaptive evolution of cancer cell quasi-species** (and of fast-evolving glycan shields of viruses like HIV, SARS-CoV-2)
- **The evolvable sugar code appears to be a fast molecular and cellular phenotype evolution mode that includes short-term evolution of acquired glycosylation with short-term selection, prior to slower genome evolution**
- Along with epigenetic multi-generational evolution, **fast glycome evolution may play an important role** prior to slower, conserved, long-term genome evolution **in unicellular/organismal evolution as well as in short-term cancer evolution**

CANCER & EVOLUTION SYMPOSIUM

Appendix to Glycosylation section:

- **Separate from cancer cell surface glycosylation, cancer metabolism needs more glucose than normal cells for rapid proliferation**
  - *Cancer anaerobic metabolism is much less efficient than breaking down sugars aerobically with oxygen. Cancer cells need as much as 40x more glucose than normal cells that metabolize with oxygen to generate the same amount of energy.*
- **We detect tumors using PET imaging of radioactively labeled sugars.**
- **Lower carbohydrate diets also can slow tumor growth in mice. Apparently, this does *not* work in cancer patients – why?**
  - *Higher carbohydrate intake stimulate insulin secretion, which not only accelerates glucose uptake by cancer cells, but also stimulates the rapid reproduction of cancer cells (and may promote chronic inflammation)*

www.sciencedaily.com/releases/2019/05/190530154220.htm, May 30, 2019: **Cancer-fighting combination targets glioblastoma**

Summary: […] researchers combined a calorie-restricted diet high in fat and low in carbohydrates with a tumor-inhibiting antibiotic and found the combination destroys cancer stem cells and mesenchymal cells, the two major cells found in glioblastoma, a fast-moving brain cancer that resists traditional treatment protocols.

*Mukherjee, P., Augur, Z.M., Li, M. et al. Therapeutic benefit of combining calorie-restricted ketogenic diet and glutamine targeting in late-stage experimental glioblastoma. Commun Biol **2,** 200 (2019). https://doi.org/10.1038/s42003-019-0455-x*