Mitochondrial involvement in cancer

Rachel Nicoll PhD

So how are we doing with President Nixon's 'War against cancer'?

President Richard Nixon signing the National Cancer Act of 1971.

- **Cancer is set to displace heart disease as the No1 killer in the Western world.**
- According to Cancer Research UK, there are over 164,000 cancer deaths in the UK each year, which is about 450 each day. **1 in 2 people in the UK born after 1960 will be diagnosed with some form of cancer during their lifetime.**
- In the 1940s, 1 in 16 had a cancer diagnosis; this had increased to 1 in 3 by 2018 (https://www.newsweek.com/are-we-winning-war-cancer-good-news-799096).
- A few doctors have admitted that any improvement in outcome is largely due to people **giving up smoking**.

What have scientists been doing all this time?

- In the mid-1970s, **scientists believed they had the answer: cancer was caused by mutations to oncogenes and therefore we could use gene-targeted therapies to treat it;** the disease will require multiple targeted (and expensive) solutions for management and prevention.
- Researchers believed that they would find 3-8 mutated genes manifesting each specific type of cancer. **What they found was that there was a random collection of mutations, with no one mutation or any combination of mutations being responsible for initiating the cancer.**
- **The mutations for each cancer type differed from person to person and sometimes even from cell to cell within the same tumour. Indeed, the American Cancer Society states that cancer is a collection of >100 different diseases**.
- **A study attempting to find the mutations driving metastasis,** arguably the most important characteristic of cancer, **found…not a single common mutation.**
- Conventional research in recent years has focused on mapping the human genome for genetic clues to cancer. The Pan-Cancer Analysis of Whole Genomes (PCAWG) has found the whole genetic code of 2,658 cancers, described in The Cancer Genome Atlas (TCGA).
- The researchers reported: 'Cancer is massively complex, with thousands of different combinations of mutations able to cause cancer', with 4-5 fundamental (but different) mutations that drive each cancer's growth. Many of these mutations occurred decades before cancer was diagnosed.

(PCAWG Transcriptome Core Group, Nature, 2020)

What about all the research funding?

- **Despite the US National Institutes of Health spending over $1 trillion dollars trying to find a cure for cancer, these gene-targeted therapies have been a great disappointment, barely improving the death rates from the 1950s.**
- **But the American Cancer Society has now admitted that genes contribute no more than 5% of our cancer risk**.
- And many of the genetic mutations turn out to be due to **modifiable epigenetic factors**.
- **Yet 95% of cancer research funding is allocated to genetic research; prevention (i.e. targeting the modifiable epigenetic factors) accounts for the remaining 5%** (Key TJ, Publ Health Nutr, 2004).

But surely there are oncogenes that cause cancer? For example BRCA1?

- **50% of women who carry the BRCA1 mutation never get breast cancer or any cancer.** Furthermore, no common mutations could differentiate invasive from non-invasive breast cancer cell lines.
- In women aged 40 or younger, who developed **early onset breast cancer**, the authors concluded that there was '**no significant difference in overall survival** or disease-free survival between patients carrying a BRCA1 or 2 mutation and those without' (Copson ER, Lancet Oncology, 2018).
- But investigation of the **purpose of the BRCA1 gene** shows that it has multiple cellular roles and is in part responsible for **repair of DNA damage**. **The BRCA1 mutation does not even cause cancer directly but through failing to repair DNA**, resulting in mutation. It is also directly involved in mitochondrial biogenesis, a mutation limiting the ability of mitochondria to reproduce.
- In addition, the only possibly commonly mutated gene in cancer is TP53, which codes for p53, a tumour-suppressor protein; a mutation in this gene allows p53 protein production to fail. But although inherited mutations in the p53 gene can increase risk for some familial cancers (such as Li-Fraumeni syndrome), most p53 defects found in cancers are not inherited and appear to arise sporadically, as do the vast majority of all cancer-associated mutations. So even inheriting gene mutations is, to some extent, a modifiable risk factor.
- Similarly, P53 is involved in numerous cellular mechanisms, including repair of the walls protecting the nucleus and transcription of a critical component of the mitochondrial ETC.

(Kenny TC, Oncogene, 2017; Seyfried T, Front Cell Dec Biol, 2015; Labbe DP, Oncogene, 2015; 'The Cancer Cascade', American Medical Research, 2004; Anand P, Pharmaceut Res, 2008)

More on the genetic approach to cancer

- There are 2 types of genetic mutation: germline (hereditary) and somatic (mutations that occur after conception as a result of diet, lifestyle and environmental exposures, which cause excessive damage to DNA). Not all somatic mutations are associated with cancer.
- However, germline genes have an average mutation rate of 0.5% every million years. Essentially, our genes have not altered since the end of the Paleolithic era.
- So they have focused on oncogene-targeted therapies and chemo- and radiotherapy designed to kill everything in sight. They work in part by creating ROS which is intended to create sufficient damage that the cancer cells opt for apoptosis.
- Even their preventive measures are centred around harmful interventions: drugs (e.g. aspirin), vaccinations (e.g. HPV) and radiation-based screening (e.g. mammograms). False positive mammograms and over-diagnosis of breast cancer among women aged 40-59 cost $4 billion annually in health care spending according to a 2015 US study (Ong MS, Health Affairs, 2016).
- And many of the genetic mutations turn out to be due to modifiable epigenetic factors. A US medical research company found that environmental toxins are responsible for 70-75% of all cancers, while infections cause 20-25% and electromagnetic radiation 5%. And that's not including poor diet and lifestyle choices.
- The mutation rate for most genes is low, making it unlikely that the numerous heritable pathogenic mutations found in cancer cells would occur sporadically within a normal human lifespan. So if mutations are such rare events, then how is it possible that cancer cells express so many different types and kinds of mutations? The genetic theory of cancer has not explained this.

Nor is cancer a disease of ageing….

- **Rates of cancer incidence are increasing in children and young people.** From the early 1980s to the early 1990s, the incidence of cancer in US children under the age of 10 rose by 37%. In adolescents and young adults, a US population-based study found that the rate of cancer increased by 29.6% between 1973 and 2015.
- **After accidents, cancer is the next most frequent cause of death in children in the US.**
- A 2016 study found that malignant brain tumours are the primary cause of cancer-related mortality in American adolescents.
- Nearly 20% of new cancer cases in the US involves someone who was a previous cancer sufferer, a rate increase of almost 300% since the 1970s.

(Mangano JJ, Int J Health Serv, 1999; Levitan D, Cancer Network, 2016; Scott AR, JAMA Netw Open, 2020)

What is wrong with conventional therapy?

- Although treatment may damage the cancer cells, it also damages healthy cells, further deplete the immune system, induce autoimmune conditions, damage DNA, eliminate healthy gut bacteria, cause intestinal permeability, lead to CVD, suppress neurological function, induce neuropathy and create inflammation and oxidative stress, all of which are cancer-promoting factors.
- Both radiation and chemotherapy are in themselves carcinogenic, with several cancer drugs, including tamoxifen, being classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC). (Early chemotherapy was derived from mustard gas, a deadly WW1 chemical weapon). They also act to reduce OXPHOS.
- Furthermore, neither surgery nor radiotherapy can destroy circulating tumour cells (CTCs) and few conventional therapies can destroy cancer stem cells (CSCs).
- None of these treatments address the root cause of the cancer, immune system deficiencies, cancer survival mechanisms or the body's functional imbalances. Cancer is a disease of the whole person, yet conventional treatments assume it is a disease of an organ or body part.
- A 1992 paper found that other than for lung cancer, there was no evidence that chemotherapy prolonged survival in patients with advanced epithelial cancer (Abel U, Biomed Pharmacother, 1992). Similarly, a review of RCTs found that the contribution of chemotherapy to 5-year survival in adults was 2.1% in the US (Morgan G, Clin Oncol, 2004).
- A 2017 UK study looked at 48 oncology drugs approved by the European Medicines Agency between 2009 and 2013 and found that most showed no evidence of benefit to survival or quality of life, even though they achieved tumour shrinkage; drug approvals are typically based on tumour shrinkage, not survival or quality of life (Davis C, BMJ, 2017).
- Radiotherapy and surgery have been around for 100 years, chemotherapy since the late 1940s.

Drug (i.e. chemotherapy) resistance 1

- When HIF-1 is inactivated, the inhibitory effect of carboplatin and etoposide on cell proliferation is significantly enhanced. Tumour cells alter their metabolism to ensure survival and evade host immune attack to proliferate. Defective apoptosis is a key form of drug resistance because anticancer treatments act in part by inducing apoptosis through activation of caspases. (Jing X, Mol Cancer, 2019)
- Autophagy in tumour cells is a double-edged sword. On the one hand, autophagy can remove misfolded proteins and dysfunctional organelles within tumour cells, inhibit the cellular stress response and ultimately prevent genomic damage, thereby inhibiting cancer. On the other hand, in the advanced stage of tumour growth, tumour cells can make use of autophagy to survive in conditions of nutrient deficiency or hypoxia. The functional inactivation of autophagy pathways results in significantly enhanced efficacy of chemotherapeutic agents. Autophagy induced by hypoxia is primarily located in hypoxic tumour regions. (Jing X, Mol Cancer, 2019)
- Substantial evidence has shown that autophagy promotes the development of multi-drug resistance (MDR). The elevated levels of autophagy detected in patients with poor prognosis indicate that autophagy can catalyse the development of MDR. Cumulative evidence suggested that autophagy, as a cytoprotective mechanism, mediated MDR, thereby protecting MDR cancer cells from apoptosis and promoting resistance to chemotherapy treatment. Autophagy-suppressors can abolish multidrug resistance. (Jing X, Mol Cancer, 2019)
- Most anticancer drugs kill tumour cells by causing DNA damage. However, cancer cells can respond with activation of repair mechanisms and signalling pathways to overcome DNA damage. Subsequently, repaired cancer cells become more resistant to chemotherapeutic treatment. (Jing X, Mol Cancer, 2019)

Drug (i.e. chemotherapy) resistance 2

- Similarly, on exposure to hypoxia, cancer cells undergo replication stress, thereby activating DNA damage and repair pathways. HIF-1α is associated with increased chemoresistance; downregulated HIF-1α can increase the sensitivity to cancer drugs. But there are many unresolved questions. (Jing X, Mol Cancer, 2019)
- The increase in glycolysis for ATP generation in cancer cells is frequently associated with resistance to therapeutic agents via upregulation of HIF. (Jing X, Mol Cancer, 2019
- The activation of the p53 pathway upon treatment with chemotherapeutic agents was found to be markedly suppressed. Moreover, the accumulation level and activity of HIF-1α increased in p53 mutant cells, thereby allowing decreased apoptotic potential and chemoresistant properties. (Jing X, Mol Cancer, 2019)
- Chemotherapy drugs have an oxygen-dependent effect on the killing of tumour cells; most have reduced efficiency under hypoxic conditions, which may be related to the reduction of free radicals. (Jing X, Mol Cancer, 2019)
- Problems with targeting drugs at the mitochondria is that CD8 cytotoxic T-lymphocytes, which are the immune system's key defence against cancer, and display remarkable metabolic similarities to cancer cells and are often damaged by the treatment. Furthermore, helper T-cell s proliferate in a similar to cancer cell proliferation and are dependent upon aerobic glycolysis and supported by mitochondrial fragmentation. (Porporato PE, Cell Res, 2018)

The ‘one mutation, one drug’ approach

- Nevertheless, it is still hoped that this genome research will lead on to developing targeted therapies and ‘smart drugs’, allowing treatment to be tailored to each patient’s unique tumour. There are now 800 smart drugs in trials.
- While these targeted drugs are a step up from the ‘kill everything in sight’ approach of traditional chemotherapy, the ‘one mutation, one drug’ approach is not working either – they are extremely expensive and barely increase survival.
- For example: Tarceva, approved 10 years ago, has significant side effects, it’s very expensive and it boosts median survival for pancreatic cancer by……10 days!

(Ju YS, Elife, 2014; Grandhi S, Hum Mol Genet, 2017)

Cancer research: Scientists seek clues to ‘how cancer is born’ BBC, 21 October 2019

- **British and American scientists are teaming up to search for the earliest signs of cancer in a bid to detect and treat the disease before it emerges.** They plan to "give birth" to cancer in the lab to see exactly what it looks like "on day one".
- But they admit this is "**like looking for a needle in a haystack" and a solution could be 30 years off**.
- **This is a tacit admission that the genomic approach has not worked.**
- But the ‘how cancer is born’ approach is also **doomed to failure. Why?**
- Every cell has the capacity to become a cancer cell under the right conditions. In fact, **we all have 75 million cancer cells in our body, every moment of every day.**
- If a cancer cell develops there are many opportunities for the immune system to catch it. **Where cancer develops and thrives, it is because immune system surveillance and targeting is defective.**
- **So they would be better off looking at the immune system!**

So let's look at how cancer actually develops

- Every cell has the capacity to become a cancer cell under the right conditions. When a healthy cell becomes damaged and mutates, it works not for the good of the body but to promote its own survival. It grows rapidly and doesn't respond to the body's natural cellular control mechanisms. Nevertheless, it takes 10-12 years on average for every cancer cell to multiply to the extent of forming a fully grown tumour. So there is a lot of opportunity for the immune system to eliminate it, if caught early.
- We all have 75 million cancer cells in our body, every moment of every day.
- Where cancer develops and thrives, it is because immune system checkpoints are defective. Failure at these checkpoints leads to apoptosis as the immune system will search out and repair damaged DNA and silence oncogenes. But if cancer cells can evade or fool the checkpoint, cancer cells will aggregate and form a tumour. They may do this by coating themselves with fibrin as they reproduce; this helps them to hide from the immune system and to literally stick together to form the tumour.
- Tumours start when a normal cell mutates into an 'immortal' cancer cell - there is much that is still not understood about this process. This cancer cell then multiplies and proliferates until it becomes a mass of cells. Once the mass reaches a certain level, it begins to establish itself in a specific organ or tissue and becomes a tumour.
- Mitochondria are necessary in tumour cell formation; tumour cells depleted of mtDNA required mitochondrial transfer to progress in growth and proliferation (Dong LF, Elife, 2017).
- As the tumour grows larger, it requires greater and greater amounts of nutrients from the blood, until it finally grows its own set of blood vessels (angiogenesis) to speed the growth process. It will also send out growth factors to signal to the rest of the body that it should aid the growth and development of the tumour.
- If left unchecked, some cancer cells will break off from the tumour and establish new tumours, elsewhere in the body (metastasis).

And how cancer metastases…

- Metastasis is defined as the development of secondary malignant growths at a distance from a primary site of cancer. It occurs when cancer cells break away from the main tumour and enter the bloodstream or lymphatic system as single migratory circulating tumour cells (CTCs) or as multicellular groupings (CTC clusters).
- The CTCs preserve primary tumour heterogeneity and mimic tumour properties. They are found in the blood of patients with solid tumours (mostly breast, prostate, brain, ovary, pancreas and colon cancer and melanoma) and function as a seed for metastasis.
- Some types of cancer tend to spread to certain parts of the body. For example, breast cancer tends to spread to the bones, liver, lungs, chest wall and brain. This new cancer is given the same name as the original cancer, for example, breast cancer that spreads to the liver is called metastatic breast cancer, not liver cancer.
- Metastasis is not the same thing as cancer invasion, which is the direct extension and penetration by cancer cells at the primary site into neighbouring tissues.
- CTCs can be used as a marker of metastasis and its progression but detection is difficult and requires highly specialised equipment. A higher number of CTCs prior to chemotherapy predicts continued metastasis and poor outcome as they have many survival mechanisms (Wang WC, BioMed Res Int, 2018; Ma S, Med Sci Monit, 2017).
- Circulating tumour cells and cancer stem cells are responsible for >95% of all metastases and cancer deaths; metastasis accounts for >95% of cancer deaths.
- There are 3 hypotheses to explain metastasis, all of which may be true:
    1. Epithelial-mesenchymal transition (EMT)
    2. The cancer stem cell hypothesis
    3. The macrophage–cancer cell fusion hybrid hypothesis.

Epithelial-mesenchymal transition (EMT) in cancer progression

- The epithelial–mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion and break through the basement membrane with increased migratory and invasive properties to become mesenchymal cells (MSCs) and enter the bloodstream through intravasation as circulating tumour cells (CTCs).
- Some cells that undergo EMT gain stem cell-like properties, giving rise to cancer stem cells (CSCs), whose properties increases their propensity to proliferate and initiate new tumours.
- In the circulation, CTCs recruit platelets for use as a physical barrier that helps protect these cells from elimination by natural killer cell-mediated lysis in the bloodstream.
- Platelets themselves can initiate EMT in cancer cells by releasing various growth factors, including VEGF for angiogenesis and transforming growth factor β (TGF-β), which enhance invasiveness and promote further metastasis. In humans, platelet counts and thrombocytosis within the upper end of the normal range have been associated with advanced, often metastatic, cancers.
- These CTCs can use the attached platelets to adhere to the new endothelium, whereupon they can exit the bloodstream at the secondary site. Here they undergo mesenchymal to epithelial transition, transforming themselves back to the original cell type to begin formation of a new tumour.
- Many studies have proposed that induction of EMT is the primary mechanism by which epithelial cancer cells acquire malignant phenotypes that promote metastasis.
- EMT also induces drug resistance and evasion of apoptosis mechanisms and confers immuno-suppression.

The stem cell theory of cancer metastasis (and mitochondrial involvement)

- The stem cell theory of cancer proposes that tumour growth and metastasis are fueled by small numbers of dedicated cancer stem cells (CSCs), which reproduce themselves and sustain the cancer. They exhibit distinctive self-renewal, proliferation and differentiation capabilities that are believed to play a critical role in cancer initiation, maintenance, progression, drug resistance and cancer recurrence or metastasis.
- Because they are stem cells, they can take up the form of any body cell, which may be why a cancer can metastasise to another form (e.g. breast cancer to bone cancer).
- Cancer stem cells display increased mitochondrial mass, membrane potential and respiration and are therefore dependent on mitochondrial activity. Increased mitochondrial mass confers stem cell-like characteristics on breast cancer cells.
- Cancer stem cells exhibit elevated rates of oxygen consumption and ROS production, as well as upregulated antioxidant capability, compared to cancer cells which are not stem cells. The upregulated antioxidant production enables them to maintain ROS levels at a lower level than in non-stem cancer cells; this enables them to proliferate, survive and resist radiotherapy and endogenous antioxidants.

(Jia D, Cells, 2018; https://med.stanford.edu/ludwigcenter/overview/theory.html)

The stem cell theory of cancer metastasis

- This implies that while tumour cells that are not cancer stem cells (CSCs) may develop into a tumour, they are not responsible for metastasis.
- Anti-cancer therapies which are designed to shrink or remove tumours do not kill cancer stem cells and consequently the tumour may grow back and will often be resistant to the therapy. In fact one breast cancer study found that multi-drug chemotherapy could actually increase metastasis risk (Karagiannis GS, Sci Transl Med, 2017).
- Cancer stem cells can then enter the circulation and give rise to metastases, in the same way that mowing the lawn chops the heads off dandelions but the seeds will be in the wind.

Dandelions Fear Me

The macrophage–cancer cell fusion hybrid hypothesis

- The fusion of tumour cells with macrophages by merging of plasma membranes is thought to lead to the development of metastasis through chemotherapeutic resistance and immune tolerance. Several studies have demonstrated that these hybrid cells are found in human cancers.
- Fusion cells are characterised by large, distinct, polymorphonuclear cancer-associated cells with dual epithelial and macrophage/myeloid properties.
- Artificial fusion of tumour cells with macrophages has been shown to lead to increased migratory, invasive and metastatic properties and may be involved in stem cell differentiation.
- This cell fusion is also a potent inducer of genomic instability.
- One study commented that ‘While primary tumours arise in a wide variety of tissues, representing not a single disease but many different diseases, metastatic cancer may be only one disease arising from a common, non-mutational event’ i.e. the macrophage-cancer cell fusion.

Factors which aid cancer development and metastasis

- Hypoxia: A typical feature in nearly all solid tumours because of the increased mitochondrial demand. The rapid proliferation of tumours outgrows their surrounding vasculature, resulting in a drop of normal oxygen levels of 2–9% to hypoxic levels of less than 2%. Hypoxia induces more aggressive, proliferative and chemo-resistant tumours and can stimulate VEGF which leads to angiogenesis. Hypoxia is especially evident in advanced metastatic cancer (Jing X, Mol Cancer, 2019; De Bari L, Cell Mol Life Sci, 2018)
- Excess acidity: Warburg and others found a correlation between cancer and low blood pH. The metabolic adaptation of cancer cells to chronic acidosis causes a shift from glucose to glutamine-fueled fermentation in tumour cells. Cancer cells can escape the immune response by causing the acidification of their environment via export of lactate. (De Bari L, Cell Mol Life Sci, 2018; Aminzadeh-Gohari S, Sem Cell Dev Biol, 2019).
- Intracellular calcium: Ca^{2+} signalling plays an important role in cancer progression by promoting proliferation, cell migration, metastasis and vascularisation and confers apoptosis resistance. Ca^{2+} crosses the outer mitochondrial membrane through the voltage-dependent anion-selective channels (VDACs). VDACs play a significant role in cancer by supporting glycolytic metabolism and preventing apoptosis. (Bustos G, Front Oncol, 2017)

The immune system in cancer

- **The immune response is the most important defence against cancer growth.** This involves development of the inflammasome through mitochondrial ROS generation and other mechanisms.
- **Immune cells of the innate immune system are continually surveying the body for cancer cells.** The main stimulatory checkpoint molecules are members of the tumour necrosis factor (TNF) receptor superfamily or the B7-CD28 superfamily. However, some cancers can protect themselves from attack by stimulating immune checkpoint targets to divert attention from the cancer cells.
- **If cancer cells are detected, cytotoxic lymphocytes and natural killer (NK) cells are recruited**, which can recognise tumour cells and control their growth.
- **Stressed or dying cancer cells can release damage-associated molecular patterns (DAMPs), which are recognised through pattern recognition receptors (PRRs)**. Once activated, **PRRs can directly kill tumour cells and/or can activate the adaptive immune system**: **cytotoxic CD8 T-lymphocytes**. These are the most specific line of defence against tumour growth and migrate into the tumour mass to kill cells, when they are known as tumour-infiltrating lymphocytes.
- **So we should be actively supporting our immune system all the time.**
- Note that **antioxidants** can inhibit inflammasome development, suggesting that indiscriminate supplementation is a mistake. Antioxidants also make chemotherapy less effective, since most work by generating so much ROS that the cell is tipped over into cell death. Antioxidants could even help cause cancer in the first place by quenching the ROS signalling that repairs the ETC.
- CAR-T cell therapy is a type of immunotherapy; CAR stand for chimeric antigen receptor. A patient's T-cells are drawn out with the blood (aphoresis) and their numbers expanded. They are then engineered to express artificial CAR receptors that specifically target cancer cell proteins and are then re-infused into the patient to attack the cancer aggressively.

(Simula L, Semin Cancer Biol, 2017; Swann JB, J Clin Invest. 2007; Pardoll DM, Nat Rev Cancer, 2012; Dunn GP, Nat Immunol. 2002)

Vaccine for cancer using Oxford/Astra Zeneca COVID vaccine technology

- Early this month came the announcement of publication of a paper showing that a viral vector vaccine made using COVID vaccine technology shows promise in overcoming cancer in mice.
- The researchers used standard immunotherapy plus the adenovirus in non-small lung cell cancer and showed an increased tumour-infiltrating CD8+ T cell response, decreased tumour size and increased survival rates compared to immunotherapy alone. Immunotherapy alone has had mixed results.
- The first human clinical trial will begin later this year.

(https://www.ludwig.ox.ac.uk/news/clinical-trial-for-therapeutic-cancer-vaccine)

Immune tolerance?

- Dr Neil Riordan (Stem Cell Therapy: A rising Tide) suggests that cancer is a ‘last ditch attempt to heal a non-healing wound’ i.e. that cancer development is working for us and not against us.
- Cancers are known to form at sites of wounds or chronic irritation. Lung cancer is an example of trying the heal the chronic lung irritation caused by smoking.
- He also talks of those who die of cancer as having developed immune tolerance to tumour cells.

The cancer stage plays a relatively unrecognised role

- Inflammation: Initially, immune activation can recruit cytotoxic lymphocytes but at later stages, chronic inflammation can suppress the immune system, promoting tumour growth and progression.
- ROS production: In early cancer stages, increased ROS is associated with decreased mitochondrial elongation and upregulation of oncogenic signalling pathways. In later cancer stages, ROS production is reduced and is associated with tumour growth and metastasis.
- Altered mitophagy: In early cancer stages, mitophagy is impaired, while at later stages it is upregulated, protecting tumour cells from mitochondrial damage which might induce apoptosis

(Simula L, Semin Cancer Biol, 2017).

So if the genome theory of cancer is wrong, where should scientists be looking?

At the mitochondria, of course!

Otto Warburg, Nobel Laureate:

1 of the only 2 Nobel Prizes awarded for mitochondrial research

- Back in the 1920s, **Otto Warburg found that cancer cells operated aerobic glycolysis** (glycolysis despite the presence of oxygen). **He hypothesised that this was due to impaired mitochondrial respiratory capacity in the cancer cells** and went on to demonstrate that deprivation of glucose and oxygen in tumour cells leads to lack of energy resulting in cell death. (Warburg O, Science, 1956)
- "Cancer, above all other diseases, has countless secondary causes…(but) **there is only one prime cause….the replacement of the respiration of oxygen in normal body cells by the fermentation of sugar**". This became known as the **Warburg Effect.**
- **But then gene theory arrived….**
- This increase in aerobic glycolysis changes the bioenergetics of the cell, reducing the activity of the TCA cycle and OXPHOS, and upregulating gluconeogenesis and lactic acid production.
- But he could not take his discovery forward because of the limitations of the scientific equipment of the time meant that very little mitochondrial research could be carried out, so it was shelved.
- Warburg's theory, however, was attacked as being too simplistic and not consistent with evidence of apparent normal respiratory function in some tumour cells. His work was not taken seriously because he was unable to explain why cancer cells would want to get their energy from inefficient fermentation if there was a reliable oxygen supply and they could produce ATP within the mitochondria. The theory also did not address the role of tumour-associated mutations, the phenomenon of metastasis, nor did it link the molecular mechanisms of uncontrolled cell growth directly to impaired respiration.

Professor Thomas Seyfried: 'Cancer as a Metabolic Disease'

- But the Warburg Effect is now being reinvestigated, principally by **Thomas Seyfried**, who wrote the (hugely expensive) book '**Cancer as a metabolic disease**' in 2012.
- Seyfried has confirmed that **nearly all cancer cells**, regardless of tissue origin, **use energy derived from aerobic glycolysis (Warburg effect), but they ferment not only glucose in the cytosol but also glutamine in the TCA cycle**, producing succinic acid through glutaminolysis. (Seyfried TN, Nutr Metab, 2010)
- **But Seyfried diverges from Warburg in finding that cancer cells can have intact mitochondrial metabolism with the ability to produce ATP normally through OXPHOS.**
- Damaged mitochondria, unable to generate enough energy for cellular survival, then send out emergency signals to the nucleus (retrograde signalling). Nuclear DNA responds and the entire complexion of the cell changes; nuclear DNA triggers the hallmark features of cancer: uncontrolled proliferation, genomic instability (leading to likelihood of DNA mutations) and evasion of cell death.
- Seyfried also described how known carcinogens (radiation, chemicals, viruses, parasites etc) do not directly cause genetic mutations. Instead they damage mitochondrial respiration, which leads to the Warburg effect. The genetic mutations resulting from carcinogens are caused mainly by excessive free radical production, as a secondary, downstream effect of dysfunctional respiration.
- Damage to mitochondria occurs first, then genomic instability, then DNA mutations. Hence the DNA mutations are a side-effect. The majority of cancer researchers disagree with this view…but history shows us how often orthodox opinion is proved wrong, turning our understanding on its head.

(Seyfried TN. Cancer as a mitochondrial metabolic disease. Front Cell Dev Biol. 2015; Seyfried TN, Nutr Metab, 2010)

More on Seyfried's work

- **Seyfried points out 3 advantages of aerobic glycolysis to cancer cells:**
  1. It produces only 2 molecules of ATP compared to the >30 from OXPHOS. BUT…. cancer cells produce ATP almost 100x faster than normal cells, so net effect is greater ATP production.
  2. The lactate produced by glycolysis is a major energy substrate in cancer: it appears to promote tumour growth, progression and metastasis, suppresses T cells of the adaptive immune system and is an extracellular and intracellular signalling molecule.
  3. Evading OXPHOS reduces ROS production, which could damage the cell and trigger apoptosis. The metabolic intermediates of aerobic glycolysis also feed the pentose phosphate pathway to facilitate macromolecular biosynthesis necessary for cancer cell growth and proliferation. Extracellularly, the lactate produced from aerobic glycolysis alters the tumour microenvironment to aid its survival.
- **Metastasis,** which accounts for most cancer-related deaths, is associated with **enhanced mitochondrial respiration and biogenesis. Inhibition of OXPHOS suppresses metastasis** in several forms of cancer. Metastasis is strongly coupled to mitochondrial activity but may to some extent be site-dependent.
- An enhanced aerobic glycolytic rate provides cancer cells with the metabolic precursors for synthesis of amino acids, nucleotides and lipids for proliferation. There is corroborative evidence that aerobic glycolysis facilitates an increased rate of glucose hydrolysis and glutaminolysis to enable cancer cells to maintain uninterrupted growth.

(Bustos G, Front Oncol, 2017; Aminzadeh-Gohari S, Sem Cell Dev Biol, 2019; Jia D, Cells, 2018; Esparza-Molto PB, Front Oncol, 2018; Ganapathy-Kanniappan S, Crit Rev Biochem Mol Biol, 2018; De Bari L, Cell Mol Life Sci, 2018; Jia D, Cells, 2018)

Is there independent corroborative evidence?

- Yes, there is **increasing evidence that cancer has metabolic origins, with upregulation of glucose transporters and glycolytic enzymes in tumour cells**.
- A 2016 meta-analysis looked at >200 studies published between 1934 and 2016 and concluded that **the most important difference between normal cells and cancer cells is how they generate energy**.
- Increased metabolism of glucose promotes several of the hallmarks of cancer, including excessive proliferation, anti-apoptotic signalling, cell cycle progression and angiogenesis. High glucose levels trigger the expression of several growth factors and also inhibit the functioning of p53, the tumour suppressing protein and inhibitor of genome mutation.
- Many tumours are characterized by low OXPHOS, but the reasons differ between cancer types. Many carry pathogenic mutations in mtDNA-encoded Complex I subunits (e.g. renal oncocytomas) or nuclear-encoded Complex II subunits (e.g. pheochromocytomas and paragangliomas); others show a reduction of all OXPHOS complexes, with a reduction of mtDNA copy number; still others have low mitochondrial mass.
- Glucose-induced insulin secretion stimulates the release of pro-inflammatory cytokines and other molecules that inhibit natural killer cells, while producing insulin-like growth factor 1 (IGF-1), a hormone that promotes tissue growth, encouraging cellular proliferation, angiogenesis, metastasis and resistance to chemotherapy.
- But one study found that cancer only results if the mutations alter mitochondrial function.

(Kenny TC, Cancer Res, 2019; Grandhi S, Hum Mol Genet, 2017; Aminzadeh-Gohari S, Sem Cell Dev Biol, 2019; Jia D, Cells, 2018; Hainault P, Curr Opin Oncol, 2012; Coussens LM, Nature, 2002; Niknamian S, Int Sci Inv J, 2016; Klement RJ, Nutr Metab, 2011; Onodera Y, J Clin Invest, 2014; Wu Y, FASEB J, 2010; Bustin SA, Trends Mol Med, 2001; Kaipparettu BA, PLoS One, 2013; Wang X, Cell Death Differ, 2015; Anand P, Pharmaceut Res, 2008)

The connections between cancer and metabolic disease

- **Obesity and diabetes are associated with incidence of many types of cancer**, including the increasingly common colorectal, prostate and postmenopausal breast cancer. It is also known that risk of pancreatic cancer is increased in patients with T2D and vice versa - i.e. the relationship is bidirectional. In addition, T2D is associated with poorer survival and poorer recurrence-free survival in cervical cancer.
- **Insulin and IGF-1 (insulin-like growth factor-1) can influence cancer initiation**, with inflammation caused by AGEs and RAGEs and release of RAGE ligands from cancer cells. Furthermore, both cancer and diabetes have been associated with abnormal lactate metabolism and high level of lactate production, which itself contributes to a higher insulin resistant status and a more malignant phenotype of cancer cells.
- **Cancer cells consume blood glucose 50 times faster than normal cells.** They do this by upregulating glucose transporters and creating more insulin receptors on the cell surface, allowing more glucose to enter. **Breast cancer cells have 3x as many insulin receptors** and colon cancer cells have 2x as many as in healthy cells.
- **A meta-analysis of 19 studies found that serum glucose was associated with incidence of both hormonal cancer and those driven by IGF-1, while an NHANES study found that blood markers of insulin and glucose metabolism were predictive of cancer mortality.**
- But some anti-cancer drugs may cause negative effects on glucose metabolism via disturbance of the insulin signalling pathway, by either causing hyperglycaemia or worsening a pre-existing diabetic condition.

(Yang X, Perspect Med Chem, 2016; Chhipa AS, Pathol Res Pract, 2019; Crawley DJ, BMC Cancer, 2014; López-Suárez A, Metabolism, 2019; Li Y, Cancer Manage Res, 2019; Chen S, Medicine, 2017; Rojas A, Carcinogenesis, 2018; Wu Y, Mediators Inflamm, 2016; Villani LA, Mol Metab, 2016; Duan W, Biomed Res Int, 2014; Parekh N, Cancer Causes Control 2010; De Bari L, Cell Mol Life Sci, 2018)

Mitochondria in healthy and cancer cells: from https://foundationformetaboliccancertherapies.com/metabolic-cancer-therapies

A healthy mitochondrion. Note the abundant looping structures inside the mitochondria (cristae). This is where all energy is produced through oxidative pathways.

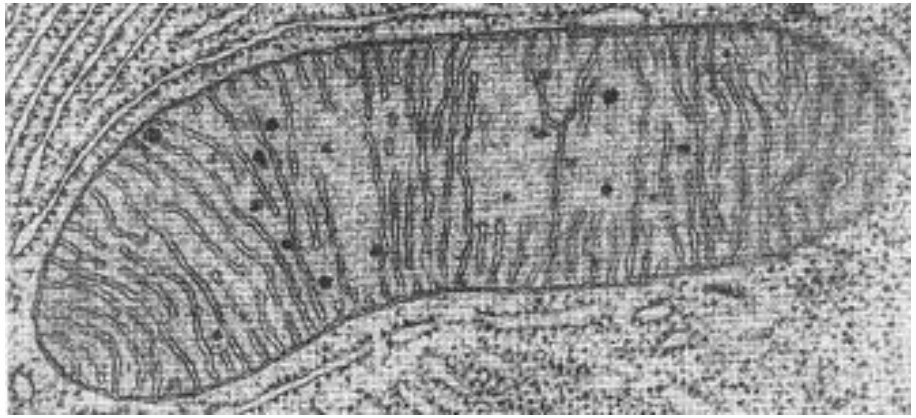
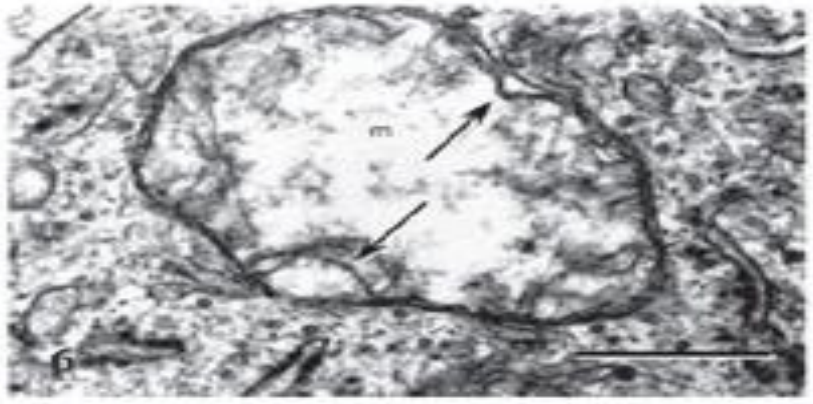
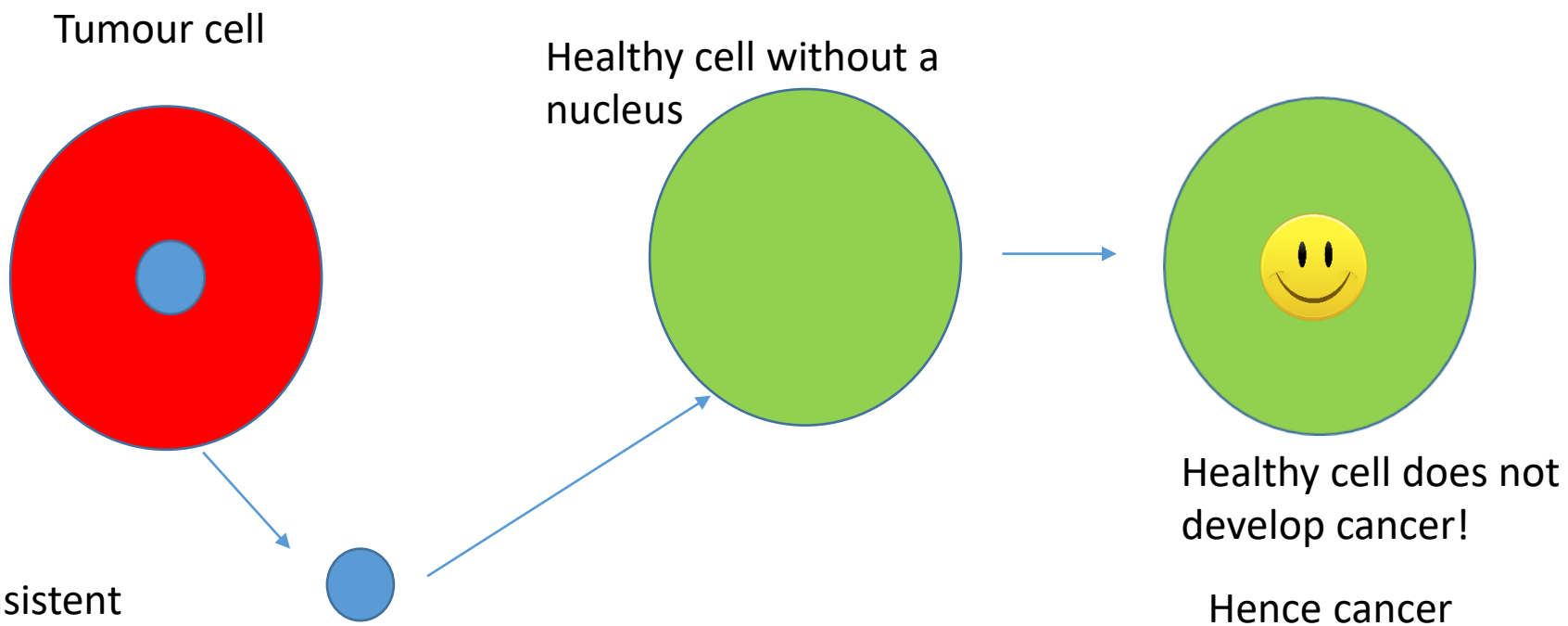


Image of a mitochondrion from a cancer cell. Note the almost compete absence of cristae.



How Seyfried disproved the genomic mutation theory of cancer by nuclear transfer (aka cloning)

When nuclei from tumour cells were transferred into healthy cells without a nucleus, the cell did not become cancerous but instead carried out normal cellular functions despite the presence of the mutant nuclear genes.

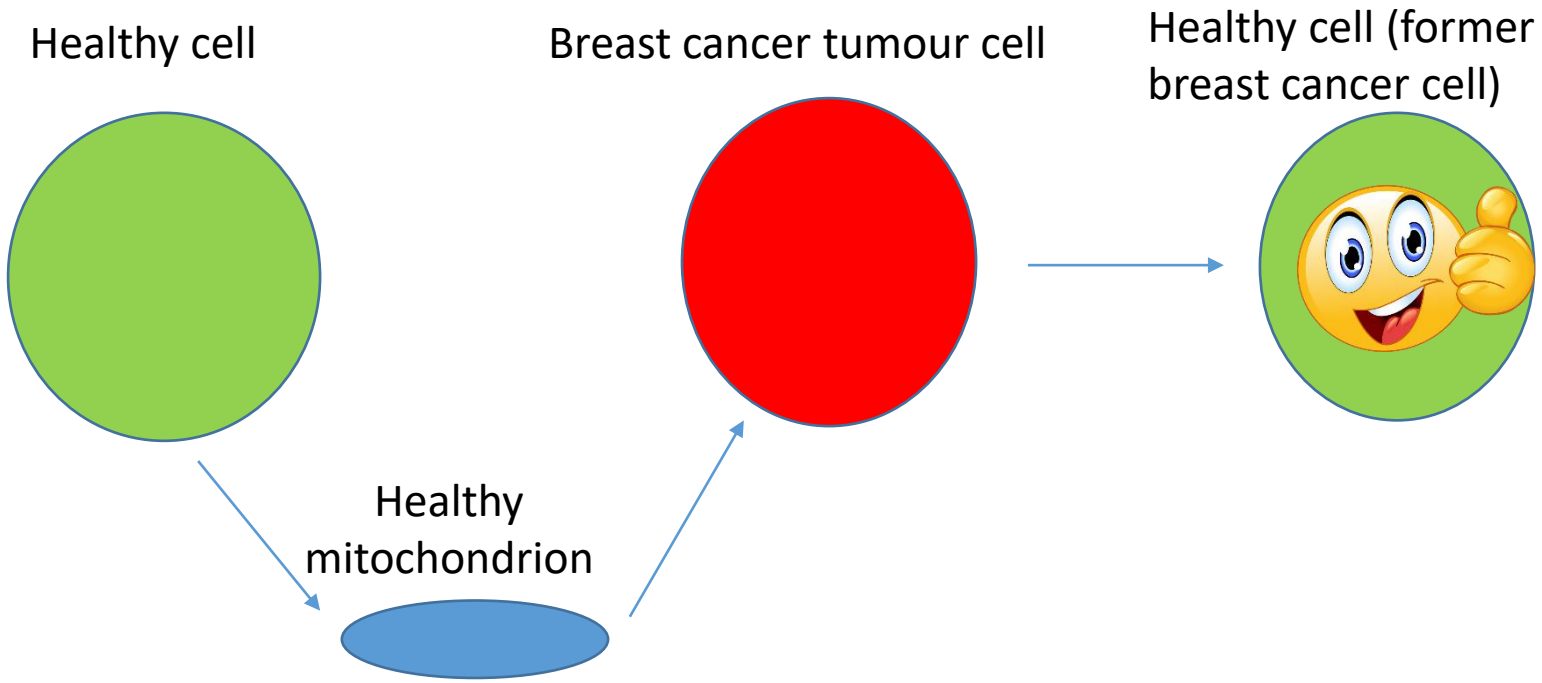


These findings were generally consistent across a broad range of tumour types, animal species and experimental techniques.

Hence cancer cannot be a genetic disease.

Seyfried shows that mitochondrial transfer can cure cancer!

When healthy mitochondria from non-cancer cells are translated into breast cancer cells, the abnormal growth and metastatic behaviour vanishes, despite the continued presence of the tumour nucleus.



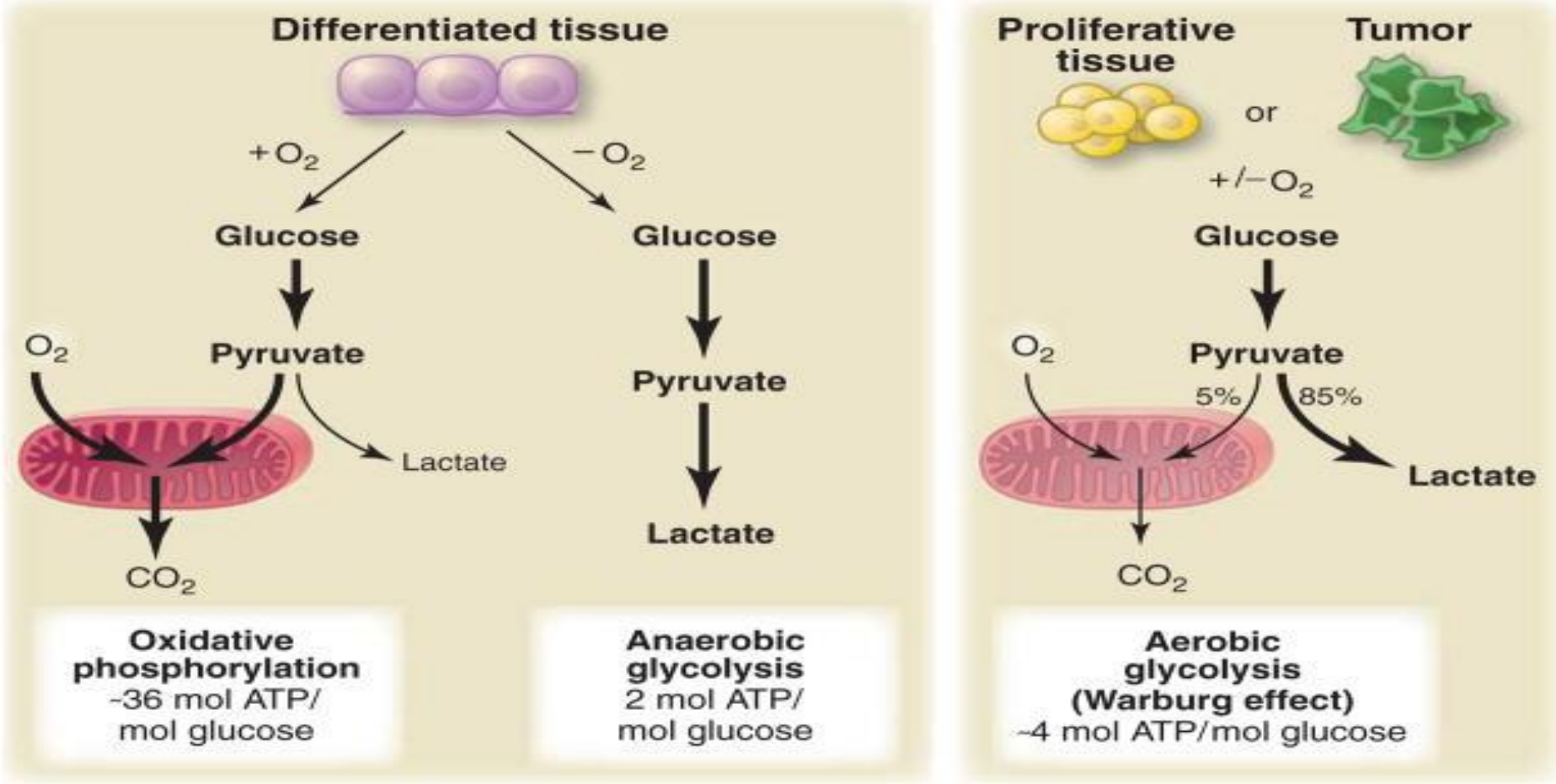
This shows that it is the mitochondria and not the nuclear genes that drive cancer. So even where there are inherited germ line mutations that cause cancer to affect the mitochondria, it is still the mitochondria that is the origin of cancer; the mutation is merely a strong risk factor.

Mitochondrial transfer

- Intact mitochondria, mitochondrial DNA or other mitochondrial components found in the circulation can be transferred between cells or tissues.
- Mitochondrial transfer between cells was carried out in the context of leukaemia, cardiac injury, acute lung injury, asthma and acute respiratory distress syndrome, through the use of tunnelling nanotube-like structures between donor and recipient cells.
- Mitochondrial transfer does not appear to have been attempted in metabolic disease, although metabolic impairment in the recipient cells was improved by mitochondrial transfer.
- But these transferred mitochondria can have a pro-inflammatory effect and elicit chemo-resistance in tumour cells and tumour proliferation.

(Montgomery M, Biology, 2019)

Comparison of respiration in healthy cells and cancer cells



From: Vander Heiden MG, Science, 2009

So what did Warburg get wrong?

- Although Warburg hypothesised that dysfunctional mitochondria were the source of aerobic glycolysis, more recent studies have found that cancer cells can have intact mitochondrial metabolism with the ability to produce ATP normally, although mitochondria may be more fragmented, which tends to facilitate glycolysis.
- Furthermore, tumours can rely on both glycolysis and OXPHOS. So the Warburg effect is now explained by altered glucose metabolism and aberrant mitochondrial signalling pathways.
- As well as fermentation of glucose, fermentation of glutamine can take place in the TCA cycle. It helps to maintain the mitochondrial membrane potential, avoiding opening of the mtPTP and the release of pro-apoptotic factors. It contributes the bulk of carbon to the TCA cycle. Glutamine is taken up by cancer cells at 10x the rate of other amino acids.
- Actively functioning mitochondria take part in tumour formation, metastasis and chemo-resistance. In fact, tumour cell mitochondria appear to be more flexible than normal cells, able to switch easily between glucose oxidation, fatty acid oxidation and glutamine oxidation, as needed, in all tumour types.
- Mutations in genes coding for TCA cycle enzymes or OXPHOS subunits have been found in some cancers, resulting in mitochondrial impairment. In these tumours, ATP is produced mainly by glycolysis.
- Where these genes are not damaged, OXPHOS is still the larger contributor to ATP production in cancer, even in a hypoxic environment, and also to provision of substrates for tumour growth and proliferation as well as signalling and survival pathways.
- This could be because cancer cells acquire different metabolic programmes at different development stages. For example, rapidly proliferating tumour cells have high glycolytic activity, which is linked to mitochondrial fission (hence fragmentation) in many cancer types. Metastatic cancer cells, however, have high oxidative metabolism, which is associated with mitochondrial fusion.

(Kitada M, Front Endocrinol, 2019; Zhu Y, J Clin Invest, 2018; Nguyen C, Cancers, 2018; Jia D, Cells, 2018; De Bari L, Cell Mol Life Sci, 2018)

How PET scans detect cancer

- Because the cancer cells are relying on glycolysis for energy, which is much less efficient than OXPHOS, it will seize any glucose that comes its way.
- If the patient is injected with radioactive glucose, it will appear on the scan being hoovered up by cancer cells and largely ignored by healthy cells. This is how cancer is imaged.
- Needless to say, this clear demonstration of cancer cell glycolysis has not prompted any revision of the gene mutation theory!

Other findings in Seyfried's work: rogue macrophages

- Seyfried has shown that macrophages can fuse with cancer stem cells, giving the hybrid cell (which he calls 'rogue macrophages') the characteristics of both stem cells and macrophages: proliferative, immunosuppressive, can enter and exit tissue and live in the bloodstream and in hypoxic environments (Seyfried TN, Crit Rev Oncog, 2013).
- Macrophages and immune cells are major glutamine consumers; many use aerobic glycolysis of glucose or glutamine during rapid proliferation, which suggests it may play a fundamental role in supporting cell growth and other anabolic reactions (Lunt SY, Annu Rev Cell Dev Biol, 2011).

Other findings in Seyfried's work: Lactate production

- In most tumours, pyruvate metabolism is altered, favouring lactate production from pyruvate. Lactate is both a major energy substrate in cancer (it appears to promote tumour growth, progression and metastasis) and an extracellular and intracellular signalling molecule. Lactate and pyruvate can also be utilised for biomass synthesis to facilitate cell proliferation.
- High lactate levels can induce a marked increase of mitochondrial mass in both normal and cancer cells. Moreover, lactate can also activate mitochondrial biogenesis.
- Lactate helps to mitigate the metabolic acidosis that arises from high rates of glycolysis. This may seem counter-intuitive since lactate is an acid (lactic acid) but the conversion of pyruvate to lactate consumes protons and additional protons are removed from the cytosol by the transport of lactate into the mitochondria. Lactate also aids the maintenance of cytosolic NAD+ concentrations and buffers variations in pyruvate concentration.
- In addition, lactate acts as an important tool for immunosuppression by tumours. The acidification of the cancer extracellular environment due to lactate export, together with proton level alteration and glucose deprivation due to the high rate of glucose consumption by cancer cells, all inhibit the activation of T cells, which suppresses their anti-tumour function. High cancer-derived extracellular lactate concentrations can also cause T- and NK-cell apoptosis.
- Lactate can migrate from hypoxic to oxygenated tumour cells to support OXPHOS. Lactate carriers are over-expressed in several cancers due to the action of transcription factors such as HIF-1. Even lactate itself can activate HIF-1 in oxidative cancers, thereby mimicking a condition of hypoxia even when oxygen is available and stimulating tumour growth and angiogenesis.
- The lactic acid is secreted into the extracellular space where it lowers pH, contributing to acidosis, which triggers angiogenesis and induces immunosuppression. Lactic acid itself can act as a metabolic fuel for cancer cells. (Romero-Garcia S, Front Immunol, 2016; De Bari L, Cell Mol Life Sci, 2018)

Characteristics of cancer cells

- Cancer cells have higher metabolic needs and antioxidant defenses compared with healthy cells. They may rely heavily on OXPHOS in addition to aerobic glycolysis to meet their energy needs and will upregulate glucose transporters. Aerobic glycolysis leads to the production of large amounts of lactate and pyruvate, causing increased acidity in the cytoplasm in cancer cells. Cancer cells exhibit extreme metabolic flexibility, able to switch substrate to promote survival and metastasis. (Urra FA, Front Oncol, 2017; Nguyen C, Cancers, 2018)
- Cancer cell mitochondria contain higher amounts of anti-apoptotic Bcl2 proteins, which activate pro-survival enzymes to evade apoptosis and resist anti-cancer drugs. Cancer cells can also upregulate enzymes such as hexokinase II, which increases lactate production, cell proliferation and resistance to chemotherapy; lactate production allows for cancer cells to maintain a slightly acidic micro-environment and enhance survival by using lactate as an antioxidant. (Nguyen C, Cancers, 2018)
- Cancer cells require a higher concentration of ROS to supplement increased proliferation rates, which may increase DNA damage and promote further mutation and tumourigenesis. Because severe oxidative stress inhibits metastatic proliferation , cancer cells have upregulated antioxidative defenses (primarily SOD) to avoid ROS-driven mtPTP opening and permeabilisation of the mitochondrial membrane, both of which lead to apoptosis. (Porporato PE, Cell Res, 2018; Urra FA, Front Oncol, 2017; Nguyen C, Cancers, 2018).
- MtDNA mutations that mildly (but not severely) affect OXPHOS and induce ROS production have been found in many forms of tumour but induction of mitophagy limits ROS production (Porporato PE, Cell Res, 2018).
- Cancer cells appear to rely much more heavily on NAMPT than normal cells (Zhu Y, J Clin Invest, 2018).
- They have an abnormal number of chromosomes (aneuploidy). Humans have 22 pairs of somatic chromosomes and 1 pair of either XX (male) or XY (female). So aneuploidy suggests that cancer cells are not properly human cells. This could explain why they have no concern for the health of the host human organism. Aneuploidy can occur when an energy-starved cell makes mistakes in mitosis through inadequate ATP production. This is hypothesised as the cause of mutations, not the genetic inheritance.

Disturbed electromagnetic activity and viral infections in cancers

- Disturbed electromagnetic activity is a fundamental feature of all cancers.
- Cancer can be initiated in a cell by short-circuiting and dampening the cellular electromagnetic field, which can occur with, *inter alia*, viruses or mitochondrial defects. This lowers cellular respiration and reverses the polarity of the water layers surrounding the mitochondria, and reduces control over chemical reactions and a higher risk of random gene mutations.
- 15-20% of all human cancers are thought to be caused by viral infections. These viruses include Epstein-Barr, hepatitis B and C, human papillomaviruses, HHV 8, human T cell leukaemia virus, and HIV-1. Oncogenic viruses have prolonged latency and the ability to establish long term persistent infections instead of killing their host cells but immune evasion. Cancer is thought to be initiated when a viral coding sequence is integrated into the cellular genome, altering host cell DNA.
- The virus also hijacks some of the host cell energy supply, thereby lowering the electromagnetic field. The electrical potential in fully functioning mitochondria is around -140mV, whereas in dysfunctional mitochondria it can be halved to around -70mV. This in turn reduces the ordering of the layers of water surrounding the mitochondria, reverses its polarity. This can lead to metastasis, as it enables a cancer cell to liberate itself from the tissue, as well as emission of electrons which increases the conductivity of the cytosol, altering intracellular communication.

(Pokorni J, Applied Sciences. 2020)

Many conventional anti-cancer drugs using mitochondrial pathways

Most commonly used chemotherapeutic drugs induce increased mtROS production and/or increased mitochondrial membrane permeability to induce apoptosis. Other mechanisms of action include inhibition of Complex 1 activity, promoting mitochondrial uncoupling and decreasing ATP production. However, drug resistance is relatively common. In addition:

- Doxorubicin has a high affinity for cardiolipin, whereby it interposes itself in mtDNA, causing disruption of genes coding for OXPHOS subunits. It also inhibits mitochondrial biogenesis in early breast cancer (Scatena C, Front Oncol, 2018).
- In breast cancer cells, tamoxifen induces cytochrome c release from mitochondria, reduces the mitochondrial membrane potential and reduces mtDNA synthesis, leading to progressive depletion of mtDNA.
- Deferiprone, an iron chelator, removes the iron needed for DNA synthesis and repair, while tetrathiomolybdenate, a copper chelator, reduces tumour angiogenesis and ATP production by inhibiting Complex IV and degrading HIF-1α.
- The NSAIDs diclofenac, aspirin and ibuprofen are Complex I inhibitors and have shown anti-cancer potential; aspirin can also inhibit Complex IV.
- Valproate, the anti-epileptic drug, and some anti-depressants cause mitochondrial toxicity and reduce respiration, thereby reducing tumourigenesis.
- Although more used for cancer pain relief, cannabinoids can also induce mtROS production, leading to apoptosis.

New or repurposed drugs to treat cancer by targeting mitochondrial pathways

- In recent years there has been considerable research on ‘mitocans’ (mitochondrial-targeted anti-cancer drugs). In general, they disrupt energy production in cancer cell mitochondria, leading to increased ROS production and activation of the mitochondria-dependent cell death signalling pathways; they can also target cancer stem cells and enhance the actions of other chemotherapeutic agents. Mitocans have shown limited adverse effects on non-cancerous cells but the long term effects are unknown. Clinical trials have shown promise and some have been licensed for use in the US.
- Antimicrobials appear to affect mitochondrial functions, probably due to the bacterial origins of mitochondria, and several (including Doxycycline and Azithromycin) can target cancer stem cells. Rapidly dividing malignant cells with high energy demands are particularly sensitive to antimicrobials but cancer cells depleted of mtDNA are resistant.
- Anti-diabetic drugs: Metformin inhibits Complex I and upregulates AMPK, leading to reduced tumour growth, increased apoptosis and reduced cancer mortality. Interestingly, metformin is a purinergic agent. Canaglifozin also inhibits Complex I and cell growth in prostate and lung cancer, while Pioglitazone inhibits mitochondrial oxygen consumption and aids anti-proliferation.
- Similarly, the sodium-glucose transporter 2 (SGLT2) inhibitors Canagliflozin, an approved medication for T2D, inhibits cellular proliferation and clonogenic survival of prostate and lung cancer cells, reducing glucose uptake, mitochondrial Complex I activity and ATP production, while activating AMPK.
- The mitochondrial fission (Drp1) inhibitor mdivi-1 has shown success in inducing proliferation arrest but its effect is dependent upon the cancer stage and there is a danger that chronic treatment may promote tumour survival.

(Ralph SJ, Recent Pat AntiCancer Drug Discov, 2006; Aminzadeh-Gohari S, Sem Cell Dev Biol, 2019; Nguyen C, Cancers, 2018; Simula L, Semin Cancer Biol, 2017; Zhu Y, J Clin Invest, 2018; Nguyen C, Cancers, 2018)

The most promising anti-cancer drug

- A US team found that cancer cell mitochondria have markedly elevated amounts of the enzyme hexokinase-2 (HK2) bound to their outer membrane. HK2 is a key enzyme in the metabolism of glucose to lactic acid in aerobic glycolysis and helps to immortalise cancer cells.
- The anti-fungal drug 3-bromopyruvate (3BP) was able to kill many types of cancer cells growing in tissue culture, eradicate tumours in animals and prevent metastasis, while leaving normal cells unharmed, through targeting HK2.
- 3BP is in fact the brominated derivative of pyruvate. The pyruvate transporter system, known to be overexpressed in cancer cells, is used to deliver bromopyruvate inside cells. (Pedersen PL, J Bioenerg Biomembr, 2012)
- Why have clinical trials for 3BP not been carried out? In Travis Cristofferson's 'Tripping over the truth', he says this is because the 'one drug kills all cancers' approach negates the genomic theory of cancer and instead tends to prove the metabolic theory. Note the association with pyruvate, aerobic glycolysis and lactate.

Cancer and the unfolded protein response (UPR)

- The UPR is a cellular response related to endoplasmic reticulum (ER) stress as a result of an accumulation of unfolded or misfolded proteins. The UPR has three aims: to restore normal cellular function by halting protein translation, degrading the misfolded proteins and activating the signalling pathways that increase production of molecular chaperones (such as heat shock protein) involved in protein folding. If these objectives are not achieved, then the UPR triggers apoptosis.
- The mitochondrion has its own UPR, occurring in the mitochondrial matrix or inner membrane. The objectives are similar to the ER UPR: induction of quality control programmes, antioxidant production, mitochondrial biogenesis and mitophagy. These are achieved in the mitochondrion through activation of SIRT3.
- The mitochondrial UPR is an adaptive stress response that functions to resolve the accumulation of unfolded proteins within mitochondria, which leads to oxidative stress. Although the functions of many mtUPR-induced genes are still unknown, it is well established that the mtUPR is activated if mitochondrial function declines to promote repair and recovery.
- As many cancers exhibit a metabolic shift from oxidative phosphorylation-dependent energy production to aerobic glycolysis-dependent energy production, researchers suggest that cancer cells rely on the mtUPR to maintain mitochondrial integrity, while inhibition of mtUPR selectively kills human cancer cells rather than non-cancer cells.

(Kenny TC, Cancer Res, 2019; Deng P, Sem Cancer Biol, 2017; Zhu Y, J Clin Invest, 2018)

So what do we need to bear in mind when dealing with cancer patients?

- **We don't want tumour cells to be healthier** – we want them to commit suicide!
- **We don't want to improve their mitochondrial metabolism** and generate additional ATP as this will fuel growth and proliferation.
- Furthermore, **cancer is metabolically extremely flexible**, can evade immune system checkpoints and has multiple survival strategies!
- **An obvious strategy is to eat zero carb**, but such is the need for glucose that cancer cells will initiate gluconeogenesis from proteins if necessary. This is what causes cachexia (breakdown of structural proteins). So there is nothing to be gained by being completely carb-free.
- Also **support the immune system as a priority**.

Therapeutic targets: far more questions than answers!

- Should we be targeting cancer cells or cancer stem cells? Studies suggest that the anti-cancer agents are different for these 2 types of cells.
- Dual therapy has shown promise in many tumour types, comprising metformin (Complex I, and hence OXPHOS, inhibitor) and 2-deoxy-D-glucose (2-DG), a glycolysis inhibitor, as they block both main sources of energy production together (Jia D, Cells, 2018).
- Complex I inhibition appears to work with anti-diabetic drugs but studies of other agents have shown that increasing Complex I activity can repress tumour growth and metastasis through regulation of NAD+/NADH redox balance (Jia D, Cells, 2018).
- Increasing ROS production could trigger apoptosis (provided the cancer cells don't upregulate antioxidant defences even further). Making the environment less acidic is effective in overcoming multi-drug resistance; studies have shown that proton pump inhibitors can increase the uptake of cisplatin in cells and enhance the role of cytotoxic agents in chemotherapy-resistant epithelial ovarian cancer (Jing X, Mol Cancer, 2019).
- Targeting sirtuins, especially SIRT3, shows promise. SIRT3 activation induces a SOD-mediated ROS reduction and decreases the high glycolysis rate in tumour cells. However, SIRT3 is already over-expressed in some forms of cancer and may be associated with chemoresistance. (Ansari A, Aging Cell, 2017; Zhu Y, J Clin Invest, 2018)
- Parkin (and hence its activator PINK1) are often downregulated in cancer cells and Parkin overexpression can slow tumour growth rate. However, some types of cancer show upregulated PINK1. (Salazar C, Cells, 2018)
- Some tumours show enhanced mitochondrial fission, and hence fragmentation, while others show enhanced mitochondrial fusion. It is not clear why. (Williams M, Front Endocrinol, 2018)

Mitochondrial therapies for cancer

- **Caloric restriction (CR)/fasting**: Dietary energy restriction naturally lowers circulating glucose levels and significantly reduces growth and progression of numerous tumour types (Raut GK, Free Radic Biol Med, 2019). Many studies show the benefits of fasting prior to chemo- or radiotherapy.
- **Ketogenic diet**: A Seyfried RCT of women with breast cancer found that a ketogenic diet for 12 weeks was associated with a significant reduction in tumour size and a regression in cancer stage compared to controls eating normally, but this only occurred in those with locally advanced cancer and not in those with metastatic cancer (Khodabakhshi A, Clin Nutr, 2020). A review found that the ketogenic diet improved blood profiles and reduced a marker of tumour progression (Chung HY, J Cancer Prev, 2017). The ketogenic diet may be ineffective for hormonal cancers (www.canceractive.com) but can reduce side effects of conventional therapy (Zahra A, Radiat Res, 2017).
- Exercise: No RCTs for treatment. Improves fitness and patient quality of life during conventional therapy (An KY, Int J Cancer, 2020).
- Hyperbaric oxygen: Reduces radiation side effects as an adjuvant therapy (Teguh DN, Int J Radiat Oncol Biol Phys, 2009).
- **Extreme temperature therapy**: In 68 patients with recurrent gastric carcinoma, radiotherapy plus abdominal hyperthermia had a significantly higher response rate than radiotherapy alone (Lyu X, Hepatogastroenterology, 2014). Also reduces hair loss in chemotherapy as an adjuvant therapy (Rice BA, Breast Cancer Res Treat, 2018).

The American Cancer Society's dietary recommendations for cancer patients

- 'Quick and easy snacks for people undergoing cancer treatment:

  cake, cookies, doughnuts, ice cream, microwaveable snacks'.

- So clearly we have a long way to go in getting the message across!

Concerns about fermentable fuels in cancer:

- Obviously we need to reduce intake of glucose (carbohydrate) and glutamine (animal and plant protein), but reducing glutamine is tricky. It is everywhere - it's the most abundant amino acid in the body.
- This suggests the ketogenic diet to force cancer cells to utilise OXPHOS, but studies have shown that fatty acids can also serve as a major energy source for cancers.
- So essentially no food is safe. Fasting? But not sustainable in the long term.
- Coffee: A meta-analysis found that a higher intake of coffee may be associated with a lower risk of prostate cancer (Chen X, BMJ Open, 2021). Gerson therapy routinely uses coffee enemas.
- Seyfried developed the 'press-pulse' cancer treatment, which involves restricting the fermentable fuels (glucose and glutamine) in a cyclical fashion to avoid causing damage to the immune system. Without glucose and glutamine, the cancer cells will starve, as they cannot use ketones. (Seyfried TN, Nutr Metab, 2017)

Vegan diets for cancer patients?

- Vegan diets have a lot of adherents but they are inherently nutrient-deficient and may include lectins and oxalates that can disrupt metabolic processes and increase gut permeability.
- And for some with a certain genotype, a vegetarian or vegan diet may increase the risk of developing cancer (Kumar S, Mol Biol Evol, 2016).
- Plant-based diets came to prominence after the China Study, which found that tofu-eating vegetarians developed less cancer than in the West. But this study, now debunked, failed to take account of the high consumption of sea vegetables and fermented foods, which would have a beneficial effect.
- Apart from anything else, a vegan diet is very high in carbohydrates, which break down into glucose.

High protein diets for cancer patients?

- Cancer patients need complete proteins, only found in meat, fish and dairy, for optimal functioning of the immune system, avoidance of cachexia, manufacture of DNA and regulation of gene expression. Proteins control almost every biochemical reaction in the body. All of these need to be supported as much as possible.
- Studies show that patients undergoing conventional cancer therapy require a 50% higher protein load.
- Why meat has such negative results in epidemiological studies: Most Americans are not eating real meat (processed meats count as meat) and the vast majority of subjects in these large population studies eat conventionally reared livestock.
- Consider what this means using the example of a chicken: it is caged for its lifetime, fed GM grain, injected with antibiotics and growth hormone and then chlorine washed. Alternatively we could eat meat from a free range organic chicken.
- But note that a very high protein diet can inhibit ketosis in some, as it is converted into glucose in the liver (gluconeogenesis).

Let's return to Thomas Seyfried

- **Whereas the oncogene theory of cancer states that mitochondrial dysfunction is an effect rather than a cause of cancer, Seyfried's approach treats cancer as 1 disease**, a disease of sick and weakened cells, which have misguidedly turned to fermentation as the only possible way to stay alive.

Using the logic of his findings, he came up with **two forms of treatment**:

- **The 'press-pulse' cancer treatment, which involves restricting glucose and glutamine in a cyclical fashion** to avoid causing damage to the immune system. However, **reducing glutamine is tricky**. It is everywhere - it's the most abundant amino acid in the body. **Cancer cells cannot use ketones as fuel**, so without glucose and glutamine they will starve. (Seyfried TN, Nutr Metab, 2017)
- Employing a **restricted calorie ketogenic diet (R-KD):** both of these cause cancer cells to die. However, healthy cells with metabolic flexibility were supercharged. Healthy cells in ketosis also have upregulated glutathione to minimise any oxidative damage.

In fact his **R-KD diet proved to be**:

- **Anti-angiogenic**, inhibiting the production of new blood vessels supplying the tumour, essential to its survival.
- **Pro-apoptotic**, facilitating orderly cell death, as opposed to the chaotic cell death ensuing from chemo- or radiotherapy, which merely increased inflammation, itself carcinogenic.
- **Anti-metastatic**: metastasis was not so widespread, likely due to downregulation of IGF-1.

Seyfried and R-KD as an adjuvant therapy

- Furthermore, fasting, the quickest route to ketosis, is shown to prepare normal cells to withstand chemotherapy, making it the ideal adjuvant therapy.
- Many studies show the benefits of fasting prior to chemo- or radiotherapy. In glioma treatment, R-KD alone slowed the growth of tumours in mice but combined with radiation treatment the result was synergistic, allowing some mice to experience a full cure. It is thought the ketogenic diet may act as an immune adjuvant.
- Seyfried also showed synergy between R-KD and the drug 2-deoxyglucose (2DG), a molecule that looks like glucose but cannot be further metabolized. It inhibits glycolysis, effectively bringing fermentation to a halt. Diet or drug alone slowed tumour growth but there was a synergistic effect when combined. He describes R-KD as preparing the therapeutic landscape, conditioning cancer cells to be killed while protecting healthy cells.
- Similarly, Seyfried trialled R-KD and the drug 6-diazo-5-oxo-L-norleucine (DON), a glutamine antagonist in mice with glioblastoma. The combination killed tumour cells while reversing disease symptoms and improved mouse survival; it also reduces oedema, haemorrhage and inflammation. The R-KD diet facilitated DON delivery to the brain and allowed a lower dosage to achieve the therapeutic effect.

(Abdelwahab MG, PLoS One, 2012; Woolf EC, J Lipid Res, 2015; Lussier DM, BMC Cancer, 2016; Marsh J, Nutr Metab (Lond), 2008; Mukherjee P. Commun Biol 2019)

Seyfried and Dominic D'Agostino: R-KD therapy and hyperbaric oxygen therapy (HBOT)

- D'Agostino pioneered the use of **hyperbaric oxygen therapy (HBOT) to generate ROS which triggered apoptosis** while correcting any hypoxia, which can be a hallmark of cancer.
- Together they tried the combined therapy on mice with highly metastatic brain cancer. The **R-KD therapy increased mean survival by 57%, but combined with HBOT mean survival increased to 78%** (Poff AM, PLoS One, 2013). They called this 'mitochondria enhancement therapy'.
- Seyfried pointed out that they had moved away from the 'no pain, no gain' approach of conventional therapy to one of health restoration: one comes out of therapy healthier than when one went in.
- They describe cancer as an ecosystem. The best way to alter an ecosystem is to change the whole environment rather than targeting a single part, as the ecosystem will adapt and long-lasting change will not occur.
- This is Seyfried's 'press-pulse' approach, with the diet gently pressing on the cancer, weakening it and rendering it vulnerable. HBOT then provides the pulse, causing stress, pushing the weakened cells over the edge. They consider that it would be as effective as radiation, without the collateral damage to normal cells.

(Seyfried TN, Nutr Metab, 2017)

ChemoThermia Oncology Centre, Istanbul, Turkey

- Employs metabolically supported chemotherapy (MSCT), which is **based on Seyfried's work, using a combination of 4 of our mitochondrial therapies (fasting, ketogenic diet, hyperthermia and hyperbaric oxygen) combined with very low dose chemotherapy.**
- Published studies (Note, these are not RCTs and there is no control group):
  - In 24 patients with stage III-IV gastric cancer, overall survival was mean 39.5 months and progression free survival was mean 36.5 months. (Iyikesici MS, Niger J Clin Pract, 2020).
  - In 25 metastatic pancreatic ductal carcinoma (stage IV) patients, median overall survival and median progression-free survival were 15.8 months and 12.9 months, respectively (Iyikesici MS, Complement Med Res, 2020).
  - In 44 stage IV non-small cell lung cancer patients, there was a 61% response rate (complete or partial), with mean overall survival of 43 months and progression-free survival of 41.0 months (Iyikesici MS, Int J Hyperthermia, 2019).

https://chemothermia.com/

Mitochondrial remedies for which there is evidence of benefit in cancer (Annex E)

- Astaxanthin
- B vitamins
- β-lapachone
- Berberine
- Butyrate
- Cannabinoids
- Capsaicin
- L-carnitine
- L-carnosine
- Coenzyme Q10
- Creatine
- Ginkgo biloba
- Ginseng
- Lipoic acid
- Magnesium
- Melatonin
- Omega 3 fatty acids
- Selenium *
- Taurine
- Vitamin A *
- Vitamin D
- Zinc *

Flavonoids and isoflavones

- Apigenin
- Baicalin/Baicalein
- Curcumin
- Epicatechin
- Epigallocatechin-3-gallate (EGCG)
- Grape seed extract
- Icaritin
- Kaempferol
- Luteolin
- Milk thistle
- Myricetin
- Naringin/Naringenin
- Nobiletin
- Quercetin
- Resveratrol
- Rutin
- Genistein
- Daidzein
- Puerarin

Vitamins and minerals with *

All essential for mitochondrial function but in excess can be highly toxic.

Both mitochondrial deficiency and excess induce mitochondrial damage.

Flavonoids

A combination works best, as this is how they arise in nature. Their individual effects complement each other.

Mitochondrial remedies for cancer: Observations

- Every one of these remedies can inflict damage on cancer cells, including cancer stem cells, while **leaving healthy cells undamaged**. This is in stark contrast to conventional chemo- and radiotherapy.
- Although there are virtually no human RCTs of these remedies on their own, many of them, particularly the bioflavonoids, have been shown to **enhance the effect of chemo- and radiotherapy in 'resistant' tumours.**
- Virtually all the remedies have been shown to **reduce the symptoms** of chemo- and radiotherapy and would be worth supplementing for this alone.
- There is **no mitochondrial remedy that is specific to any one type of cancer**, as Seyfried would predict. They can all induce apoptosis in cancer cells and prevent growth, proliferation and metastasis.
- **Caveat**: Vitamin A, B vitamins, taurine and selenium deficiency and excess can induce cancer. Capsaicin, gingko biloba and phytoestrogens, particularly if taken for long periods, can induce some cancers.

Mitochondrial remedies for cancer in humans: the top 4

- Berberine: 600mg/day to prevent colorectal adenoma recurrence.
- Melatonin: 20mg/day may improve survival in patients with solid tumours
- Resveratrol: doses as low as 500mg/day could reduce tumour cell proliferation in colorectal cancer.
- A combination of 60mg/day quercetin and 1140mg/day curcumin for 6 months decreased polyp number and size in familial adenomatous polyposis.

(Mixed results for human RCTs of omega 3 fats and vitamin D)

The Sunday Telegraph

Government may intervene to curb dangerous 'cancer cure' propaganda 9 August 2020

- ‘Ministers discuss expanding the Cancer Act to police medically unproven procedures and bring in tighter regulations on social media’ in order **to protect people from ‘fake cancer treatments’ and ‘dodgy practitioners’.**
- What this actually means is that our government is seeking to prevent cancer patients from choosing natural health treatments, contravening both their basic human rights and civil liberties.
- And what about the scores of people who die from properly prescribed and administered conventional treatments such as chemotherapy and radiation? Where is the legislation to protect them?
- The Cancer Act in the UK currently prevents the advertising of treatments for cancer patients that are outside of the mainstream medical system. But this could be extended to prevent cancer sufferers from consulting non-medical health professionals or engaging in any additional diagnostic testing.
- And if this approach succeeds with cancer, what disease is next?