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Mitochondria and the evolutionary roots of cancer

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Abstract
Cancer disease is inherent to, and widespread among, metazoans. Yet, some of the hallmarks of cancer such as uncontrolled cell proliferation, lack of apoptosis, hypoxia, fermentative metabolism and free cell motility (metastasis) are akin to a prokaryotic lifestyle, suggesting a link between cancer disease and evolution. In this hypothesis paper, we propose that cancer cells represent a phenotypic reversion to the earliest stage of eukaryotic evolution. This reversion is triggered by the dysregulation of the mitochondria due to cumulative oxidative damage to mitochondrial and nuclear DNA. As a result, the phenotype of normal, differentiated cells gradually reverts to the phenotype of a facultative anaerobic, heterotrophic cell optimized for survival and proliferation in hypoxic environments. This phenotype matches the phenotype of the last eukaryotic common ancestor (LECA) that resulted from the endosymbiosis between an α-proteobacteria (which later became the mitochondria) and an archaebacteria. As such, the evolution of cancer within one individual can be viewed as a recapitulation of the evolution of the eukaryotic cell from fully differentiated cells to LECA. This evolutionary model of cancer is compatible with the current understanding of the disease, and explains the evolutionary basis for most of the hallmarks of cancer, as well as the link between the disease and aging. It could also open new avenues for treatment directed at reestablishing the synergy between the mitochondria and the cancerous cell.

1. Introduction

Exploring the evolutionary nature of cancer might impact our understanding of the disease and the development of treatments, and could also reveal new fundamental aspects of the evolution of metazoans (Merlo *et al* 2006, Hartl *et al* 2010, Srivastava *et al* 2010, Davies and Lineweaver 2011). Cancer cells are dysfunctional, but they are not weakened or impaired. In fact, cancer cells are highly proliferating and invasive, and difficult to eradicate. Hence, while cancer might ultimately cause the death of an organism, cancer cells appear to be successful, self-sufficient, competent and highly adapted, the trademarks of evolution. Cancer disease is widespread among animals (e.g. Scharrer and Lochead 1950, Black and Baumann 1991, Nagy *et al* 2007), and it appears to be an evolutionary phenomenon deeply rooted in the metazoan branch of the tree of life. Oncogenes have been traced all the way back to some of the early metazoans (Hartl *et al* 2010), and the basic principles that define cancer are strikingly antagonistic to the basic principles that define multicellularity (Srivastava *et al* 2010). If cancer has indeed evolutionary roots, then the question arises: how deep are those roots?

Mitochondrial dysregulation is one of the most common hallmarks of cancer, and it was given a central role in earlier models of cancer disease (Warburg *et al* 1927, Warburg 1956). This role is being recognized again in more recent studies of hypoxia, oxidative stress and metabolism in tumour cells (Gogvadze *et al* 2008). Mitochondria are organelles that evolved from the endosymbiosis of two unicellular organisms, in one of the defining moments in the evolution of eukaryotes.

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The link between cancer, mitochondria and the evolution of eukaryotes is the basis for our hypothesis that cancer cells revert to a phenotype akin to the last eukaryotic common ancestor (LECA).

2. The endosymbiotic nature of the eukaryotic cell

The endosymbiotic hypothesis states that the eukaryotic cell evolved from the symbiotic merging of two or more prokaryotic cells (Margulis 1970, Martin *et al* 2001). While the exact sequence and the timing of the endosymbiotic event are unknown, the universality of the mitochondria among eukaryotes (with the exception of some anaerobic eukaryotes, see below) suggests that it was one of the early organelles acquired by LECA. Phylogenetic reconstructions based on mitochondrial DNA (mtDNA), and metabolic and evolutionary considerations have traced the mitochondria back to an ancestral α-proteobacteria (Gray 1992, Gray *et al* 1999, Martin *et al* 2001), while the host cell appears to have been an archaebacteria (e.g. Cotton and McInerney 2010).

It has been estimated that the initial endosymbiotic event leading to the mitochondria initiated about 2 Gyr ago (e.g. Sicheritz-Pontén *et al* 1998). This timing overlaps with the main period of oxygenation of the Earth's atmosphere, which began 2.4 billion years ago with a rapid rise in O_2 levels up to 1% pO_2 during the Proterozoic, and a second rise up to 21% pO_2 in the Phanerozoic, approximately 600 million years ago (Fike *et al* 2006, Canfield *et al* 2007). Based on geochemical evidence oceans must have been anoxic below the photic zone between 2.4 and 1.8 Gyr ago (Arnold *et al* 2004, Lyons and Reinhard 2009), and euxinic (anoxic and sulfidic) between 1.8 Gyr and 750 Ma (Poulton *et al* 2004). Hence, the symbiosis of the α-proteobacteria and the archaebacteria likely took place in the anoxic and sulfidic ocean, but allowed the new organism to survive in an increasingly oxygenated environment, owing to the capability of the α-proteobacteria to utilize oxygen by means of its respiratory chain.

In fact, the acquisition of new metabolic pathways by the host was one of the key benefits resulting from the endosymbiotic event. Phylogenetic reconstructions of genes and enzymes involved in the metabolism of modern eukaryotes indicate that both the oxidative pathway and the glycolytic pathway were inherited from the eubacteria, not the archaebacteria (Fothergill-Gilmore and Michels 1993, Müller *et al* 2012). Key enzymes in the respiratory chain of mitochondria responsible for oxidative phosphorylation (OXPHOS) are codified by mtDNA, and the same respiratory chain complex is found in modern bacteria. Therefore, OXPHOS in eukaryotes is clearly a trait inherited from the α-proteobacteria endosymbiont. Genes and enzymes of the glycolytic pathway in modern eukaryotes also have homologues among the eubacteria, but not among the archaebacteria; however, it is yet unclear whether they were acquired from the α-proteobacteria endosymbiont, or from other eubacteria, for example through horizontal gene transfer (i.e. Müller *et al* 2012).

Irrespectively of its exact origin, cytosolic glycolysis is a universal trait of eukaryotic cells, and therefore it must have been acquired before the divergence of all modern groups. OXPHOS is common to all aerobic eukaryotes, and it occurs exclusively within the mitochondria. Notable exceptions are the anaerobic eukaryotes, which contain modified versions of mitochondria—the hydrogenosomes and the mitosomes. Hydrogenosomes are involved in anaerobic metabolism and produce hydrogen, acetate, carbon dioxide and ATP, whereas the function of mitosomes is still a matter of debate, and appears to be related to the biosynthesis of cytosolic Fe–S proteins. These organisms were once thought to represent the earliest descendents of aerobic eukaryotes. However, the realization that both hydrogenosomes and mitosomes are modified mitochondria, and the distribution of anaerobic eukaryotes in all main groups of eukaryotes, but not necessarily at the bottom of the eukaryotic tree (see Müller *et al* 2012), suggest instead that these groups also evolved from a mitochondrion-containing ancestor common to all eukaryotes, but do not necessarily represent ancient eukaryotic lineages.

Hence, while still fragmented, the picture that emerges regarding the earliest eukaryotic cells is as follows: approximately 2 Gyr ago a unicellular organism originated from the symbiosis of two anaerobic cells (an α-proteobacteria endosymbiont and an archaebacteria host). The α-proteobacteria was capable of respiring oxygen and likely also of fermentative metabolism (Gabaldón and Huynen 2003). The original metabolism of the archeabacterial host is still not understood (Kurland and Anderson 2000; but see Martin and Müller 1998), but it must have been replaced at an early stage by the glycolytic pathway of the eubacteria. As a result, a symbiotic organism capable of cytosolic glycolysis and oxygen respiration emerged. This symbiotic organism was likely free living and motile, and occupied anoxic or hypoxic niches in the water column or in sediments. This likely was the common ancestor to modern eukaryotes (LECA), and subsequent evolution lead to its divergence into the modern eukaryotic groups.

3. Life after LECA

Evidence shows that the final assimilation of the α-proteobacteria endosymbiont into an organelle involved a massive reduction of its genetic code, and the assignments of new physiological functions by the host (cf Kurland and Anderson 2000). As a result, the genome of present-day mitochondria little resembles that of its α-proteobacteria ancestor. Clear examples are the highly modified hydrogenosomes and mitosomes, but even the genotype and the phenotype of modern mitochondria have been substantially modified. As thoroughly explained by Kurland and Anderson (2000), the evolution of the mitochondria from an aerobic prokaryote to an organelle is better understood as the evolution of its proteome. Studies of the mtDNA from the yeast *Saccharomyces cerevisiae* revealed that about 50% of mitochondrial proteins do not have eubacterial nor archaeal homologues, and only 1/10 are unequivocally of α-proteobacteria origin (Karlberg *et al* 2000). It was further concluded that a large fraction of the original genes in the α-proteobacteria ancestor were lost in the process of endosymbiosis, or transferred to the host nuclear

genome, while a small fraction of genes were recruited by the α-proteobacteria endosymbiont from the nuclear genome (Karlberg *et al* 2000). A similar process of genetic information reduction and transfer to the nucleus is observed in mammalian mitochondria, which contain over 1500 proteins but only 13 of these proteins are encoded by mtDNA (Nunnari and Soumalainen 2012). The dual nature of the mitochondrial proteome shows a clear correspondence with protein functionality. Mitochondrial proteins with bacterial homologues (i.e. those that have been conserved from the original α-proteobacteria ancestor, like the cytochrome c oxidase) seem to be involved in translation and energy metabolism, whereas mitochondrial proteins with eukaryotic homologues are typically associated with transport and regulatory functions (Karlberg *et al* 2000, Kurland and Anderson 2000).

Hence, the coordinated functions between the nucleus and the mitochondria in modern eukaryotic cells are the result of progressive, step-wise and complex genomic and morphologic modifications since the original endosymbiotic event. This coordinated action is best exemplified in complex, multicellular organisms, where the mitochondria play a key role in cell regulation, metabolism and programmed cell death. The question then arises: what would happen if the mitochondria become dysregulated, and the original endosymbiotic contract is rendered obsolete? In the following, we argue that one of the consequences is cancer.

4. Cancer: a phenotypic state akin to LECA triggered by mitochondria dysregulation

Mitochondrial DNA is prone to damage and mutations induced by reactive oxygen species (ROS) that are formed in the mitochondrial matrix as a result of aerobic respiration. In fact, the majority of intracellular ROS in eukaryotic cells are by-products of mitochondrial respiration (Gogvadze *et al* 2008). The mitochondrial electron transport chain contains several redox centres that can leak electrons to molecular oxygen, serving as the primary source of superoxide production in most tissues. ROS can be persistent and cause damage to mitochondrial enzymes, lipids and mtDNA. Mutations of mtDNA genes that codify for subunits of the cytochrome oxidase complex (COx) can in turn exacerbate the oxidative stress in the mitochondria by altering the electron transport components, which further compromises the normal electron flow. This can lead to positive feedback loops whereby the formation of ROS causes mutations to mtDNA, whose expression leads to further oxidative stress. Additionally, the mitochondrial genome is predisposed to mutation as a consequence of the sheer number of replications of the genome in the life cycle of a cell (Lee *et al* 2000, Van Remmen *et al* 2003, Cook and Higuchi 2012), and elevated oxidative stress can also mutagenize nuclear DNA (nDNA). There are repair mechanisms to alleviate the negative effects of ROS byproducts on the mtDNA, such as protective proteins and small molecules that scavenge ROS or sequester metal ions; however, damages to mtDNA are largely cumulative and irreversible, and there appears to be a direct cause–consequence relationship between aging and the accumulation of mutations in the mtDNA (Cortopassi and Arnheim 1990, Jessie *et al* 2001, Greaves *et al* 2012).

Mutations and polymorphisms in mtDNA, and mitochondrial dysregulations are common in cancer cells (Wallace 2005, Singh *et al* 2005, Greaves *et al* 2012) and other diseases (Cook and Higuchi 2012). Incidence rates of cancer and metastasis dramatically increase with age (American Cancer Society 2011), and the depletion of the mitochondrial genome content is a common characteristic in the progression of a variety of cancers (Lee *et al* 2005), including ovarian (Wang *et al* 2006), gastric (Wu *et al* 2005), hepatocellular (Yin *et al* 2004), renal cell (Simonnet *et al* 2002), prostate (Moro *et al* 2008) and breast cancers (Naito *et al* 2008). There is further evidence that mutations accumulate in the mtDNA, in part, as a result of long exposure to environmental factors such as ultraviolet light (Birch-Machin and Swalwell 2010), bacteria, viruses (Machado *et al* 2010) and tobacco (Prior *et al* 2006, Tan *et al* 2008), which are also carcinogenic. Additionally, studies have shown that some cancers are caused by mutations in nuclear-encoded mitochondrial enzyme genes (Brandon *et al* 2006), and it is now well established that mitochondrial defects caused by oxidative damage to mtDNA and nDNA are linked to neoplasm (Brandon *et al* 2006).

Whether mutations in mtDNA are a cause or a consequence of cancer is still a matter of debate. However, it has been shown that the invasive phenotype of human cells depleted of mtDNA is reversed when exogenous wild-type mitochondria are reintroduced in the cell (Singh *et al* 2005). This suggests that mutations in mtDNA at least play a fundamental role in the progression of cancer, if not its initiation. Seyfried and Shelton (2010) conclude that non-critical damages to OXPHOS can potentially initiate the path to a malignant cancer. In fact alterations of the mitochondrial respiratory chain are one of the first manifestations of mitochondrial dysregulation during cancer. During pre-malignant stages of cancer and during the evolution of the disease itself, the correct expression of COx appears to be impaired. COx is the only cellular structure to be encoded from both the nuclear genome and the mitochondria (Cook and Higuchi 2012). COx is comprised of subunits synthesized from mtDNA as well as nDNA, and as such its synthesis must be tightly regulated both by the mitochondria and the nucleus. In normal differentiated cells, mitochondrial and nuclear encoded subunits are expressed in a orchestrated fashion; however, specimens of human prostate cancer tissue display a ubiquitous shift in the ratio of nuclear encoded COx subunits IV, Vb and VIc relative to mitochondrial encoded COx subunits I and II, and these shifts appear very early in premalignant conditions, and increase in magnitude as the carcinoma cells become invasive (Herrmann *et al* 2003). Further, the frequency of deficient COx I subunits has been found to increase with age (concurrent with cancer and mtDNA damage), and COx I deficiency was also found to be associated with neoplasia (Bernstein *et al* 2010).

Based on the above, we hypothesize that key mitochondrial functions become dysregulated as a result of mtDNA and nDNA mutations. The dysregulation of the mitochondria breaks the original endosymbiotic contract

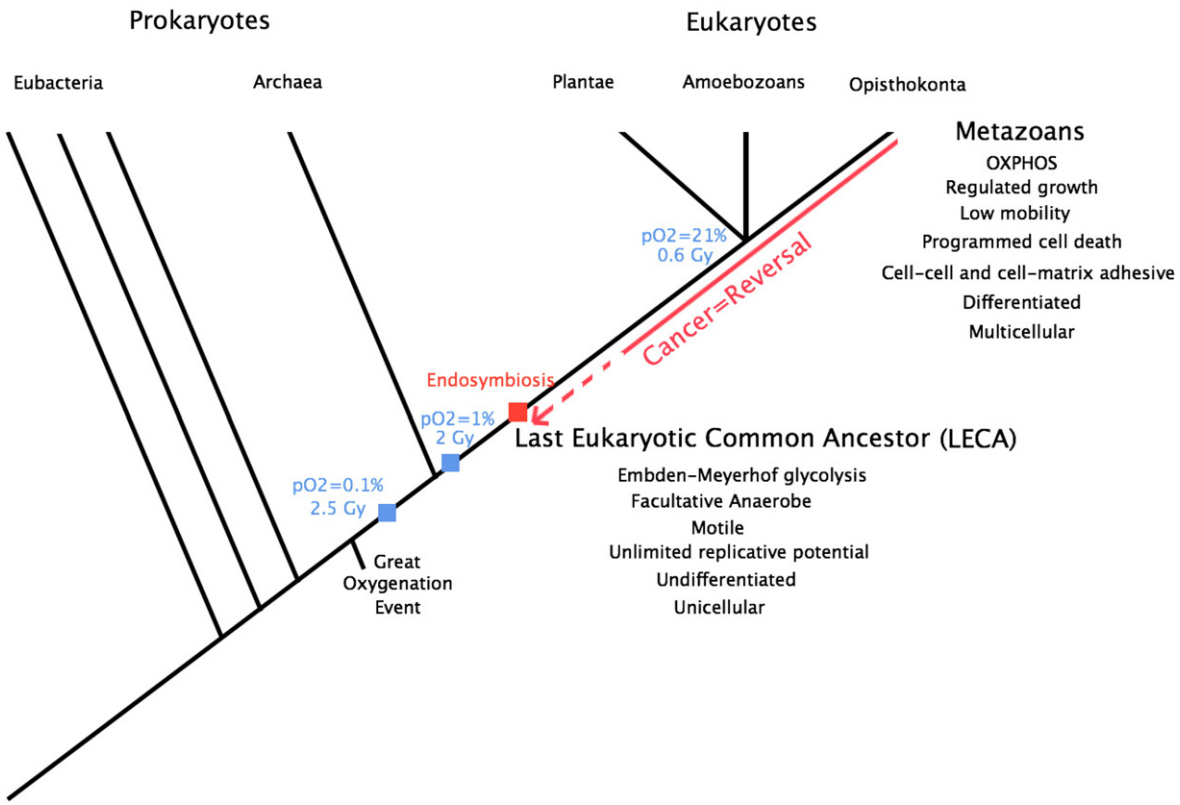

Figure 1. Schematic diagram showing the tree of life with the main subdivisions, and the relative occurrence of events leading to the emergence of the LECA. In our atavistic model, the progression of cancer results in phenotypes that are reminiscent of LECA. This evolutionary regression is triggered by the dysregulation of the mitochondria.

established before the divergence of LECA. However, this does not necessarily result in cell death. Instead, the somatic cells can revert to a phenotype that is independent of mitochondrial functions, similar to the early stages following the endosymbiotic event. Davies and Lineweaver (2011) also described tumours as an atavistic state similar to colonies of proto-metazoans about 1 billion years ago. In their atavistic model, an ancient *toolkit* of genes is activated in cancer cells, which leads to uncontrolled cell proliferation (tumours akin to primitive multicellular organisms). Our model brings this atavistic state further back in time to the early stages of the evolution of LECA, about 1.8 Gyr ago, but the mechanism could be analogous to that proposed by Davies and Lineveawer (2011). Namely, when mitochondrial activity is dysregulated or impaired, a cell can unleash a series of adaptative steps to ensure survival by activating an ancient *toolkit* of genes that are otherwise subdued in normal cells. It is tempting to suggest that oncogenes could represent this ancient *toolkit*, and their activation through random mutations deviates the cell from its normal regulated phenotype, towards an ancient proliferative phenotype more reminiscent of prokaryotic organisms, until the most advanced (malignant) cancer cells represent the lifestyle of LECA. Under this atavistic model, the evolution of cancer within one individual can be viewed as a recapitulation of the evolution of the eukaryotic cell from fully differentiated cells to LECA: (1) a switch to a facultative anaerobic metabolism to fuel cell growth and division (aerobic glycolysis); (2) the growth of colonies of differentiated and stem cells with only a moderate division of labour (early stage tumours); (3) growth of highly proliferative colonies of stem cells with replicative immortality (late stage tumours); and (4) a free living, unicellular, motile (stem) cell, with a facultative anaerobic metabolism and with replicative immortality (metastatic cell) (figure 1). Hundreds of millions of years of evolution since LECA imply that the putative ancient *toolkit* that becomes activated in cancer cells after the dysregulation of the mitochondria is not an exact copy of LECA's genotype. Hence, malignant cancer cells might be better viewed as a phenotypic replicate, but not an exact genotypic copy, of LECA.

5. The phenotype of cancer cells and LECA

5.1. Aerobic glycolysis: the metabolic phenotype of cancer cells and LECA

An increase in cytosolic glycolysis is one of the first manifestations in the early stages of cancer (i.e. the so-called Warburg effect; Warburg *et al* 1927, Warburg 1956), to the extent that high rates of glycolysis are used as a diagnostic tool for cancer. Cancer cells convert glucose to lactate, even though cytosolic glycolysis produces ATP less efficiently than mitochondrial oxidative phosphorylation. Importantly, this occurs even in the presence of oxygen; hence, the glycolytic metabolism of cancer cells has been termed aerobic glycolysis. It is important to note that high levels of glycolysis

in cancer cells do not necessarily imply that OXPHOS is impaired, and in fact, studies have shown tumour mitochondria do respire and produce ATP (e.g. Moreno-Sanchez *et al* 2007, Seyfried and Shelton 2010).

As mentioned earlier, LECA consisted of two metabolically self-sufficient symbionts: the proto-mitochondrion (an α-proteobacterium) capable of oxygen respiration (Gabaldón and Huynen 2003), and the host, a facultative anaerobic, heterotrophic eukaryote capable of cytosolic glycolysis. Hence, the metabolism of LECA must have been largely based on aerobic glycolysis for the synthesis of biomolecules (cell proliferation). Interestingly, LECA and cancer cells are not the sole examples of cells capable of aerobic glycolysis. Many eukaryotes live in fully oxic habitats but without using O_2 for OXPHOS. For example, Trypanosomes in the mammalian bloodstream synthesize all of their ATP through substrate-level phosphorylation in glycolysis despite ample oxygen and glucose availability. They excrete an oxidized end product (pyruvate) instead of a reduced end product (lactate), and their mitochondria uses O_2 as the terminal electron acceptor but without mitochondrial ATP synthesis (Müller *et al* 2012). Hence, aerobic glycolysis is not an aberration of cancer cells, but an actual metabolic phenotype among protozoa.

The switch to glycolysis in cancer cells is an adaptation to facilitate the uptake and incorporation of nutrients into the biomass needed to produce new cells, the same strategy used by bacteria in the presence of nutrients to drive cell proliferation (Vander Heiden *et al* 2009). One implicit prediction of our atavistic model is that as cancer progresses, and cancer cells become more similar to LECA, glycolytic metabolism will become more prevalent at the expense of OXPHOS, and the metabolism of cancer cells will be mostly directed towards synthesis of biomolecules and cell proliferation.

5.2. Hypoxia: the optimal environment for cancer cells and LECA

One possible explanation of the Warburg effect is that cancer cells revert to cytosolic glycolysis in response to tumour hypoxia, but observations suggest that cancer cells use glycolysis before exposure to hypoxia (Vander-Heiden *et al* 2009). Instead, hypoxia appears to be a later stage phenotype of cancer cells that also favours cell proliferation and invasiveness (metastasis) (Seidel *et al* 2010). As glycolysis fuels tumour growth, portions of the tumour tissue become hypoxic due to poor vascularization. For example, a median pO_2 of $<$15 mmHg has been found in many types of human solid tumours, in contrast to a median of $>$35 mmHg in respective adjacent normal tissues (Kim *et al* 2009). Generally speaking, hypoxia induces a wide range of biological changes that contribute to the evolution of malignant tumour cells, and for that reason hypoxia is used as a prognostic factor for advanced disease condition.

Tumour hypoxia is readily explained in our atavistic model of cancer. LECA was a facultative anaerobe capable of survival in environments with elevated O_2 thanks to its endosymbiotic host, but optimally adapted to hypoxic niches. Indeed, molecular, fossil and geochemical evidence indicate that LECA lived between 1.9 and 1.6 Gyr ago, when oceans were incompletely ventilated, with sulfidic water masses lying beneath moderately oxygenated surface waters (Parfrey *et al* 2011). Hence, the growth of a tumour might be seen as an attempt of cancer cells to recreate the optimal proliferative conditions of LECA. Interestingly, the idea that cancer cells create their own hypoxic niche for optimal proliferation also has parallels among the protozoa. *Trichomonas vaginalis* cells in laboratory cultures with O_2 break down glucose to pyruvate without cell proliferation. The free energy generated is not conserved by the NADH oxidase nor as ATP, and the reaction proceeds until the medium is free of O_2, at which point the cells commence normal growth, channelling carbon flux into O_2-sensitive pathways, but in an environment that they have themselves made anaerobic to favour enzyme functionality (Müller *et al* 2012). Finally, mathematical simulations of tumour growth show that under conditions of short oxygen supply, the morphology of the tumour is invasive, with small fingers or lobes protruding from the edge of the tumour body (Anderson *et al* 2006). This morphology of growth resembles that of colonies of anaerobic organisms, which produce typical flat spreading colonies with large spreading fans or lobes that grow out from the margins (Jones *et al* 1980).

5.3. Apoptosis: a selection mechanism in tumours?

Apoptosis is a special mechanism of programmed cell death (PCD) that involves the mitochondria. During apoptosis, mitochondrial proteins called caspases are released into the cytosol. Caspases bind to apoptosis inhibiting proteins and deactivate them, allowing apoptosis to proceed. The mitochondrial cytochrome c is also released into the cytosol during apoptosis and unleashes a series of biochemical reactions that stimulate apoptosis. In cancer cells a series of oncogenes are involved in arresting apoptosis and ensure cell survival and proliferation (i.e. oncogene Bcl-2). Blc-2 proteins are over-expressed in cancer cells and prevent the formation of the mitochondrial apoptosis-induced channel (MAC) which plays a central role in the onset of apoptosis. On the other hand, apoptosis-promoting proteins such as p53 are also typically mutated in cancer cells, which also favours tumour advance. However, apoptosis is not completely inhibited in tumours, and some oncogenic changes promote, rather than suppress apoptosis (Lowe and Lin 2000).

The evolutionary role of apoptosis is not well understood. Certainly, regulation of apoptosis must have been a key evolutionary step towards multicellularity, and given the involvement of the mitochondria it likely dates further back, to at least the earlier eukaryotic cells (Kroemer 1997, Koonin and Aravind 2002). It has been hypothesized that apoptosis could have been a mechanism originally evolved by the α-proteobacteria endosymbiont to eliminate the host cell under stress (e.g. nutrient starvation) and metabolize its remains (Frade and Michaelidis 1997). In other words, the mechanism of apoptosis in metazoans may be a vestige of evolutionary conflicts within the eukaryotic cell (Blackstone and Green 1999), which later evolved into a mechanism to regulate cell

growth in multicellular organisms. In this scenario, apoptosis becomes a strong selective mechanism for the proliferation of cells according to their fitness. Stressed or unhealthy cells are eliminated at the expense of well-adapted cells. The result is a more rapid expansion and proliferation of the colony.

Accordingly, under the atavistic model apoptosis in cancer cells ought to be a mechanism of selection based on the fitness of individual cells, rather than a mechanism to regulate multicellular growth. A corollary of this is that apoptosis within tumours ought to increase tumour proliferation and tumour fitness by selecting cancer cells that are better able to proliferate within the tumour. As mentioned above cancer cells are highly glycolytic and tumour malignancy increases with hypoxia. If glycolytic metabolism and hypoxia are positive indications of *tumour fitness*, then a direct prediction of the atavistic model is that apoptosis rates should decrease, but glycolysis rates and hypoxia levels should increase, as tumours progress, and particularly within secondary tumours.

5.4. Undifferentiation, replicative immortality and growth: the proliferative phenotype of cancer cells and LECA

It has been recently demonstrated that normal differentiated cells can dedifferentiate into stem-like cells (Cancer Stem Cells, CSCs), and that oncogenic transformation enhances the spontaneous conversion, so that non-stem cancer cells give rise to CSC-like cells (Chaffer *et al* 2011). In addition, tumour hypoxia not only favours the proliferation of tumour cells but it may also prevent differentiation and thus may maintain tumour cells in an undifferentiated 'stem cell-like' state (Kim *et al* 2009). Hence, the evolution of tumours appears to result in a positive feedback that favours the growth of stem-like cells, which in turn leads to further tumour growth, more hypoxia and more CSCs. This positive feedback loop might be reinforced by the apoptotic selection of cancer cells according to their fitness and their capability to grow and metastasize. One of the consequences of the proliferation of CSCs is that tumours become clonal in nature, similar to colonies of unicellular prokaryotes, albeit a certain degree of genotypic variability is recognized in tumours, which could explain tumour progression and resistance to treatment (i.e. Park *et al* 2010). CSCs also display high levels of telomerase activity, which confers cells with immortality by stabilizing chromosomes after cell division. This allows CSCs to grow uncontrollably, and to regenerate and renew the tumour tissue. Together, these observations indicate that as cancer progresses the dominant phenotypes are directed at cell proliferation, survival and growth, and cells within the tumour become clonal. In that respect the phenotype of an advanced tumour bears many similarities to a colony of anaerobic organisms or a biofilm.

5.5. Activating invasion and metastasis

Malignant cancer cells acquire the ability to metastasize: abandon the original tumour and spread to other locations to start a new colony. Metastasis relies upon cell motility, which results in the invasion of neighbouring connective tissue and entry into lymphatics and blood vessels (intravasation). For metastasis to occur malignant cells have to detach from adjacent cells, digest the extracellular matrix (EM), become motile and attach to the new site. Interestingly, tumour cell motility is characterized as solitary amoeboid movement (Condeelis *et al* 2005, Kedrin *et al* 2007), another example of atavistic condition of a free-living, facultative anaerobic cell. It has been hypothesized (Li *et al* 2007) that the ability of a tumour to metastasize is an inherent property of a subset of CSCs (mCSC, metastatic cancer stem cells). Single CSCs can initiate and sustain cancer growth giving their unlimited replication potential and their resistance to apoptosis. The nature of CSCs also allows them to adapt and survive in a foreign environment better than differentiated and highly specialized cancer cells (Li *et al* 2006). These are all remarkable characteristics of CSCs that approximate them to LECA: a free-living, motile, facultative anaerobic organism capable of proliferative growth and replicative immortality.

6. Does cancer recapitulate eukaryotic evolution?

Under the atavistic model, the evolution of cancer within one individual can be viewed as a recapitulation of the evolution of the eukaryotic cell from modern differentiated cells to LECA. In other words, the evolution of tumours might be seen as a movie of the evolution of the eukaryotic cell, played in reverse and at high speed, whereby the more aggressive phenotype a tumour achieves, the more it resembles LECA. Broadly speaking, tissues of normal differentiated cells would be on one end of the cancer spectrum. Tumours comprising cancer stem cells would represent self-organized assemblages of clone cells with only a moderate division of labour (an intermediate stage in the evolution of multicellularity). Metastatic cancer stem cells would represent LECA: a free-living, motile, facultative anaerobic organism capable of proliferative growth and replicative immortality. Similar 'ontology recapitulates phylogeny' models of cancer have been proposed recently (Davies and Lineweaver 2011, Fernandes *et al* 2012). Placing cancer in such evolutionary context could transform our understanding of the disease and offer new therapeutic possibilities (Merlo *et al* 2006, Davies and Lineweaver 2011). Conversely, a thorough understanding of the physiological and morphological traits of cancer might be relevant to reconstruct the early origins of eukaryotes, and their road to multicellularity.

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