Stress-induced polyploidy shifts somatic cells towards a pro-tumourogenic unicellular gene transcription network

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Hypothesis: Polyploidy enables access to transcriptional networks of unicellular organisms, which in the absence of tumour suppressors provides immortality and resistance from treatment for cancer cells

Abstract Theories of cancer are central to our understanding of biology and receive frequent refinement. Here, we propose a link between key aspects of the atavistic theory of cancer and the capacity of polyploidy to access transcriptional networks of unicellular organisms. Polyploid cells are known to display greater capacity for adaptation to environmental challenge than their diploid counterparts. Whole genome duplication (WGD) induced by environmental crisis is crucial for facilitating the genetic bias of speciation and for providing the long-term increase in genetic and biological complexity. Somatic tumour cells appear to reverse this process in response to stress. Our recent studies reveal that polyploidy is cooperatively linked by cellular stress to stemness, dedifferentiation and a shift towards the transcriptional networks typical of unicellular organisms. We hypothesise that when cells undergo polyploidy they enter a transcriptional continuum enabling rapid epigenetic adaptation to environmental challenge followed by clonal selection of genetic differences. For tumour cells, in the absence of tumour suppressors this polyploidy-induced stemness state provides access to the transcriptional network of eukaryotic precursors, whose immortality and survival fitness are supported by their a-sexual

ploidy life cycles. This process can be equated to a reversal along the phylogenetic tree of evolution providing the single-cell autonomy and immortality that are fundamental hallmarks of cancer.

Keywords: cancer, resistance to drugs, tetraploidy-induced stemness, descent to protists.

Introduction - What is cancer?

Our current understanding of cancer is at a critical juncture with the prevailing somatic mutation/clonal selection theory being challenged by more recent epigenetic theories (Marusyk et al. 2012; Baker 2015). The first proposes that stochastic driver mutations are the causative agents of cancer, while the second posits that cancer derives principally from a pre-programmed epigenetic basis (Kauffman 1971; Huang et al, 2009). Both theories are supported by solid experimental data and given the elusive nature of causation in complex systems we envisage both will sooner or later be integrated into a single, 'field-like' theory (Barabási et al., 1999).
In fact this integration is already present to some extent in the oldest embryological theory of cancer (Conheim 1877-80; Pierce and Wallace 1971; Erenpreiss 1993) and, more recently, in cancer stem cell (CSC) theory (Cabrera et al., 2015). Both are unified by the concept of cancer as 'development gone awry" (Soto et al. 2008) which provides optimism that it may be corrected and reversed (Pierce and Wallace 1971; Telerman and Amson 2009; Bizzarri et al., 2011; Erenpreisa et al., 2015; Sell et al., 2015; Pattabiraman and Weinberg 2017; Zhou et al., 2017).

Here, we develop these ideas further, integrating both the 'epigenetic' and 'genetic' aspects of cancer in order to derive a new conceptual framework. Within this, we must take into account the result of our nearly 50 year-long 'war on cancer". Irrespective of an increasing arms race, with ever more sophisticated new therapeutics, we have to acknowledge that so far "cancer has won" (Hanahan 2014). The principle lesson learnt from this sobering observation is that the strongest and most general feature of cancer cells is their ability to withstand extinction (Walther et al., 2015), and therein be highly adaptive to cellular stress. The adaptive strategies deployed by cancer cells are multi-faceted and varied and importantly do not necessarily imply a mutational basis, as indicated from two independent lines of evidence:

1. An increasing number of non-genotoxic carcinogens have been discovered (Benigni et al., 2013, 2015).

2. Large, increasingly sensitive, next generation sequencing endeavours such as the cancer genome project reveal an increasingly large proportion of cancers without mutations, causing some to question the pre-requisite for mutation in oncogenesis (Versteeg 2014; Gatenby 2017).

Nevertheless, irrespective of whether genetic mutations are cause or effect, it is clear that they are active players in oncogenesis. Principal evidence for this is seen in the frequency with which cancer cells lose TP53 function, likely being mutated or inactivated/bypassed in all cancers (Kastan 2007). TP53 is the guardian of genome fidelity, regulating cell cycle checkpoints and diploidy (Aylon and Ohren, 2011). It follows that chromosome instability and mutability rather than certain mutations *per se*, are the principle instruments of adaptation to stress. Clearly, beneficial mutations may be further selected by Darwinian processes, but likely these are secondary. Rather, we postulate that epigenetic plasticity is a prerequisite for cancer cell initiation, fitness, and progression. The question that follows is how the epigenetic control of the transcriptome in normal and cancer cells is different, allowing the latter to withstand and overcome a wide range of treatments? Tetraploidy may provide a clue.

Stress switches cells to tetraploidy associated with stemness

Tetraploidy is gaining recognition as a crucial step towards cancer (Castedo et al., 2010; Davoli and de Lange, 2011; Van de Peer et al., 2017). Most solid tumours are pre-disposed to polyploidy and aneuploidy which correlate with resistance to anti-cancer treatment and poor prognosis (Erenpreisa and Wheatley 2005; Ganem et al., 2007; Coward and Harding 2014). Pre-cancerous tetraploidy is a recognized characteristic of Barrett oesophagus, the precursor of oesophageal cancer, associated with a chronic cycle of acid reflux damage and wound healing (Walen 2015); tetraploidy is induced in peripheral lymphocytes by the tumour promoting phorbol ester (Vinogradov et al., 1991). Moreover, aging normal fibroblasts can become tetraploid expressing the ESC transcription factor Nanog, following genotoxic stress (Huna et al., 2011). The same was seen (i.e. tetraploidy and induction of an ESC-like signature with OCT4A, NANOG and SOX2 expression) in TP53 mutant but not wild type lymphoma cell lines (Salmina et al., 2010); as well as in breast cancer cells (Lagadec et al., 2012). The irradiation/drug – induced polyploidy facilitates a reversal of senescence and an increased survival of the polyploid cells before they go back to diploidy and mitosis (Illidge et al., 2000; Ivanov et al., 2003; Puig et

al., 2008; Vitale et al., 2011; Mirzayans et al., 2017; Erenpreisa and Cragg, 2013; Erenpreisa et al., 2017). As such, illicit tetraploidy resulting from the loss of TP53 cell cycle control appears to mediate the CSC~ESC conversion of tumour cells.

In turn, stemness is characterised by "poised" chromatin, allowing rapid switching on/off of the key developmental genes (Bernstein et al., 2006; Chaffer et al., 2013; Pisco and Huang, 2015) and by the thermodynamics of self-organisation, which permits low probability events, such as cell fate change, to occur (MacArthur and Lemischka 2013; Mojtahedi et al., 2016). Thus, here the two essential mechanisms bridging the survival capacity of tumour cells to ontogenesis are evident: 1) epigenetic plasticity (stemness) and 2) overcoming the barrier to tetraploidy. This whole genome duplication requires further discussion.

The role of whole genome duplications (WGD) in the evolution of species

Whole genome duplications (WGD) are known to be a driving force of species evolution, facilitating the branching of the phylogenetic tree by generating the raw material for the new genes that promote speciation and genetic and biological complexity (Van der Peer et al., 2017). Eventually, the new genomes are fixed during the return to diploidy and Darwinian selection (Kondrashov 1997; Kondrashov and Kondrashov 2006). However, WGD also has short-term effects that are essentially epigenetic and adaptive (Comai 2005, Otto 2007; Conant 2010) with implications for cancer microevolution (Gerlinger et al. 2014; Yant and Bomblies, 2015; Vazquez-Martin et al., 2016; Van de Peer et al., 2017). Having established these 'internal' relationships between polyploidy and stemness, we now consider the link with the phylogeny of cancer.

The atavistic theory of cancer

Several evolutionary theories of cancer describe the concept of a cancer state or attractor being present but remaining unused in the memory of differentiated somatic cells, which can be awakened by cancer mutations. This cancer attractor (Kauffman 1971) is located near the top of the metaphoric Waddington epigenetic hill (Waddington 1956), but at the foot of the ontogenetic tree (Huang 2009) and is thus pre-programmed (Huang et al, 2009; Erenpreisa 2014; Vinnitsky 2014; Erenpreisa et al., 2015). Recent work has indicated that the stem cell transcriptional

program expressed by aggressive cancers (Ben-Porath et al., 2008; Erenpreisa et al., 2011, 2015) likely exploits ancient pathways (Weinberg 2012), and so Haeckel's concept that 'ontogeny repeats phylogeny" (1866) is acquiring a renaissance through a deeper molecular understanding. The phylogenetic reversal of cancer cells towards early protists was suggested previously (Erenpreisa and Wheatley 2005; Erenpreisa et al., 2005; Erenpreisa and Cragg 2008; 2013; Niculescu 2016) and formulated by some authors as the atavistic theory of cancer (Davies and Lineweaver 2011; Vincent 2011, 2012; Arguello 2011; Davies 2013; Lineweaver et al., 2014). Interestingly, in human cells two main coexpression nexus of different evolutionary origin were revealed; a widely expressed basic-eukaryotic (unicellular; UC) network and a more narrow, metazoan nexus. A higher proportion of the basic eukaryotic genes were observed to be expressed in cancer tissues (Vinogradov 2010). Furthermore, a recent study of seven different human tumour types revealed enrichment of genes belonging to the UC strata (prokaryotes and first eukaryotes) in cancer cells, while normal cells shared more genes with later multicellular (MC) strata (Trigos et al., 2017). Among the UC genes expressed in tumours there are those providing basic cell functions such as ribosomal synthesis, cytoskeleton, glycolysis, RNA catabolism as well as those providing PI3K and MEK signalling. The latter is potentially carcinogenic through activated *H-ras* (Erenpreisa and Cragg 2013). The mostly disbalanced „ancestral" genes relate mainly to DNA instability in the cell cycle, regulate glycolysis and enrichment with cytoskeleton proteins, which provide endurance to hypoxia and mesenchymal-type motility. These genes are suppressed in normal epithelial cells but overexpressed in cancers. The surprising enrichment in human cancer of the 1st prokaryotic phylostratum (Trigos et al., 2017) may be associated, in addition, with the resurrection of prokaryotic endosymbiosis (Lineweaver et al., 2014; Diaz-Carballo et al., 2015; Sterrer 2016). Furthermore, Trigos et al., 2017 also revealed a higher negative correlation between the expression of UC and MC genes in tumours than in normal tissues, suggesting the two nexus may be mutually exclusive. The found regularities were common at least for seven types of cancer, including lung adenocarcinoma, lung squamous cell carcinoma, breast cancer, prostate adenocarcinoma, liver cancer, colon cancer, and stomach cancer. In turn, Wu and colleagues (2015) demonstrated that drug resistance of tumour cells develops coincident with a significant change in expression of the UC genes, which unlike the MC genes remain protected against hyper-mutability. Thus, epigenetic descent along the phylogenetic tree, rather than inherent mutation or even acquired mutations, is likely

responsible for the adaptation of tumour cells to stress and their resistance to treatment. Next, we should consider how such an epigenetic shift to unicellularity can be achieved and whether this phylo-stratiographic shift in tumours is associated with tetraploidy and the resulting induction of stemness. For this purpose we chose to examine the c-myc-targeted transcriptomes of normal polyploid mammalian cells. (Figure 1)

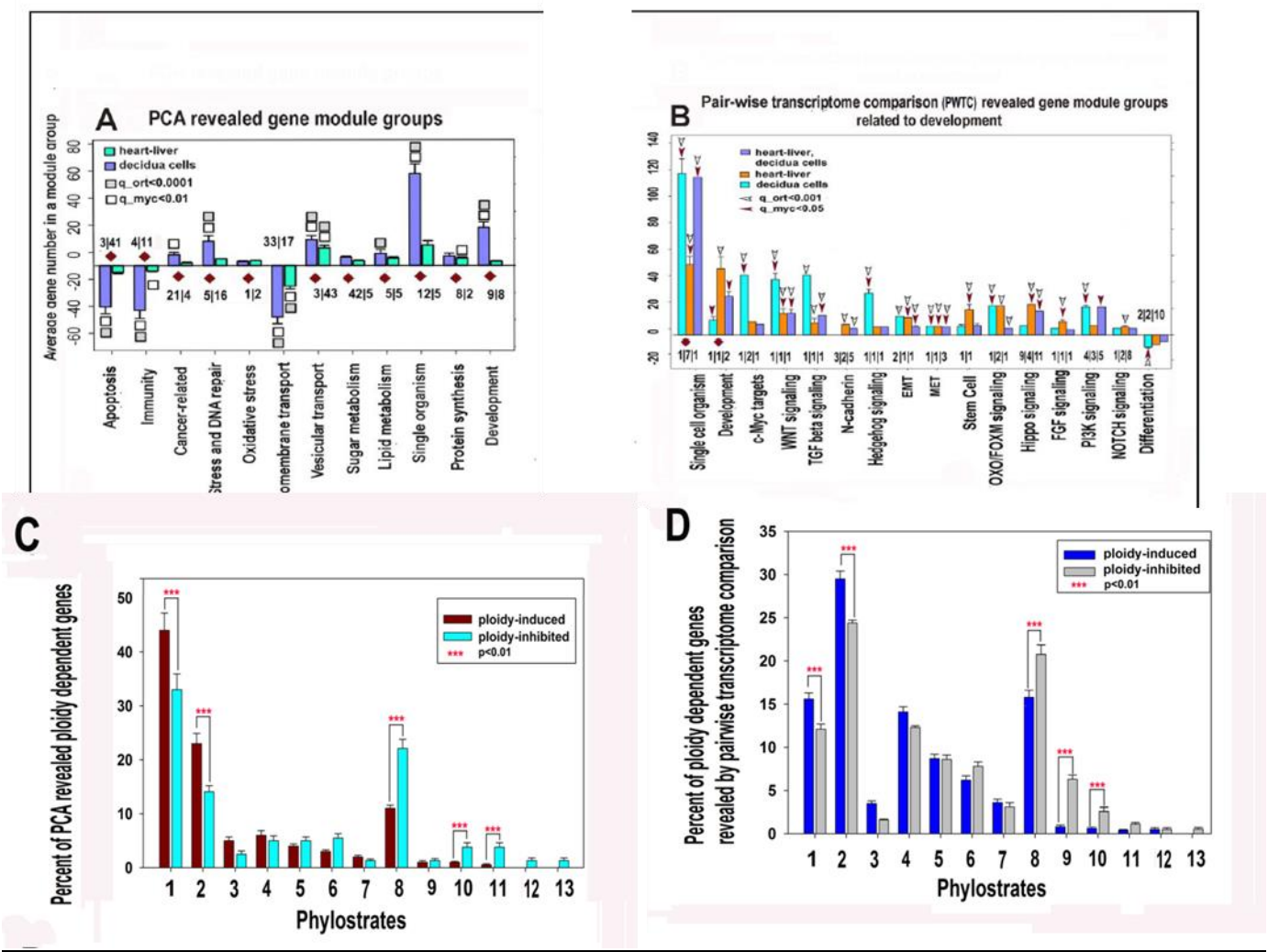

Fig. 1. Polyploidy shifts cells towards expression of UC gene modules with the aid of activated *c-myc* in human and mouse heart, liver and placenta (A, B). (A) Ploidy *c-myc* associated gene module groups revealed by principle component analysis (PCA). (B) Pairwise cross-species comparison. Both methods identify gene modules related to stress, stemness, UC organisms, glycolysis, and epithelial to mesenchymal transition (EMT) related to cancer. Red diamonds show module groups confirmed by both methods (PCA and pair-wise transcriptome comparison). Republished from Vazquez-Martin et al., 2016.

(C,D) Distribution of c-myc-dependent tetraploidy-regulated genes in human heart and mouse liver by age index determined by phylostratiography. (C) Ploidy regulation assessed by PCA and (D) by pairwise transcriptome comparison (Vazquez-Martin, et al., 2016). The gene age index data were taken from Trigos et al., 2017. This figure illustrates that polyploidy shifts the age index balance of the expressed genes from the metazoan phylostratum towards the phylostratum of unicellularity (1-3 stratum).

Down the phylogenetic tree: function of over-expressed c-*myc* and down-regulated p53 in stress-adaptive tetraploidy

c-*myc* over-expression elicits tumours, whilst its suppression affords their regression (Morton and Sansom 2013). Importantly, besides being a developmental proto-oncogene (Erenpreiss 1993), it is also one of the Yamanaka factors required for iPSC reprogramming (Takayashi and Yamanaka 2006; Buganim et al., 2013). A versatile transcription factor, c-myc also coordinates DNA synthesis with mitosis but when overexpressed it induces polyploidy (Li and Dang, 1999). Importantly, all of these functions are performed without mutation of myc (Erenpreiss 1993) and arise from simple changes in expression, such as may be achieved during polyploidy. Comparison of *myc*-targeted transcriptomes in diploid and polyploid cells in normal mammalian organs of heart, liver, and placenta (Vazquez-Martin et al 2016) shows that tetraploidy enhances the transcription of genes involved in the stress response: adaptation to hypoxia by enhanced glycolysis, enhanced metabolism and protein turnover, stemness and epithelial-mesenchymal transition (EMT but also MET), while differentiation, apoptosis and immunity become suppressed (Fig.1 A, B). Moreover, polyploidy enhances the connectivity of c-myc with the complementary oncogenic H-ras hub (for more details see Vazquez-Martin et al., 2016). Interestingly, the UC gene module was the biggest (by the number of involved genes) among c-myc induced genes. The phylo-stratography of this data based on the polyploidy-related c-myc induced and inhibited genes is presented in Fig.1C, D. The data show that also in normal cells c-myc related polyploidy enhances expression of the early phylostrata (UC) genes and suppresses the genes of higher animals. The negative correlation between the expression of UC and MC genes for polyploid versus diploid cells observed in normal tissues as seen on Fig. 1C,D, is similar to the higher negative correlation seen between the expression of UC and MC genes in tumours compared with normal cells as revealed by Trigos et al. (2017). These data support our concept that tetraploidy through c-myc-related adaptation to stress introduces an unconstrained

stemness continuum allowing cells to access the UC transcriptional networks associated with cancer. A sharp suppression by polyploidy of MC genes occurring from the strata 8 (bony fishes) may be explained by the fact that from this stratum all three members of the *TP53* gene family become active and conserved (Belyi et al., 2010). Thus, the two "most serious addictions" of cancer cells to overexpression of the proto-oncogen *c-myc* (Morton and Sansom 2013) and inevitable loss of tumour suppressor *TP53* function (Kasten 2007; Aylon and Ohren, 2011), both associated with loss of cell cycle control (resulting in polyploidy), appear phylogenetically linked to the loss of the evolutionary constraints of tissue and species-specificity (Huang et al., 2005; Huang 2009; Giuliani 2010; Reuveni and Giuliani 2012 a,b**).** It posits cancer as a disease of phylogenetic reprogramming. The question arises, how the immortality of tumour cells is related to their ploidy-associated atavism?

Reproduction of tumour cells by an asexual life-cycle-like process is also borrowed from unicellular organisms

Replication immortality is a hallmark of cancer (Hanahan and Weinberg, 2011). What is its origin? Sexual reproduction sets limits of species borders (Davison 1998). However, UC contrary to MC organisms, are firstly, immortal (Morgan 1903) and secondly, developed a-sexual life-cycles, which in many cases serve as a bridge between transient polyploids and sexual diploids (Raikov 1982; Kondrashov 1994, 1997; Erenpreisa et al., 2005; Heng 2007; Freeling 2017). It seems that cancer cells (particularly becoming resistant to drugs) employ this strategy supporting their immortality through a UC-like a-sexual ploidy 'life cycle' as suggested by us earlier (Erenpreisa and Cragg 2007, see a modified schematic in Fig.2).

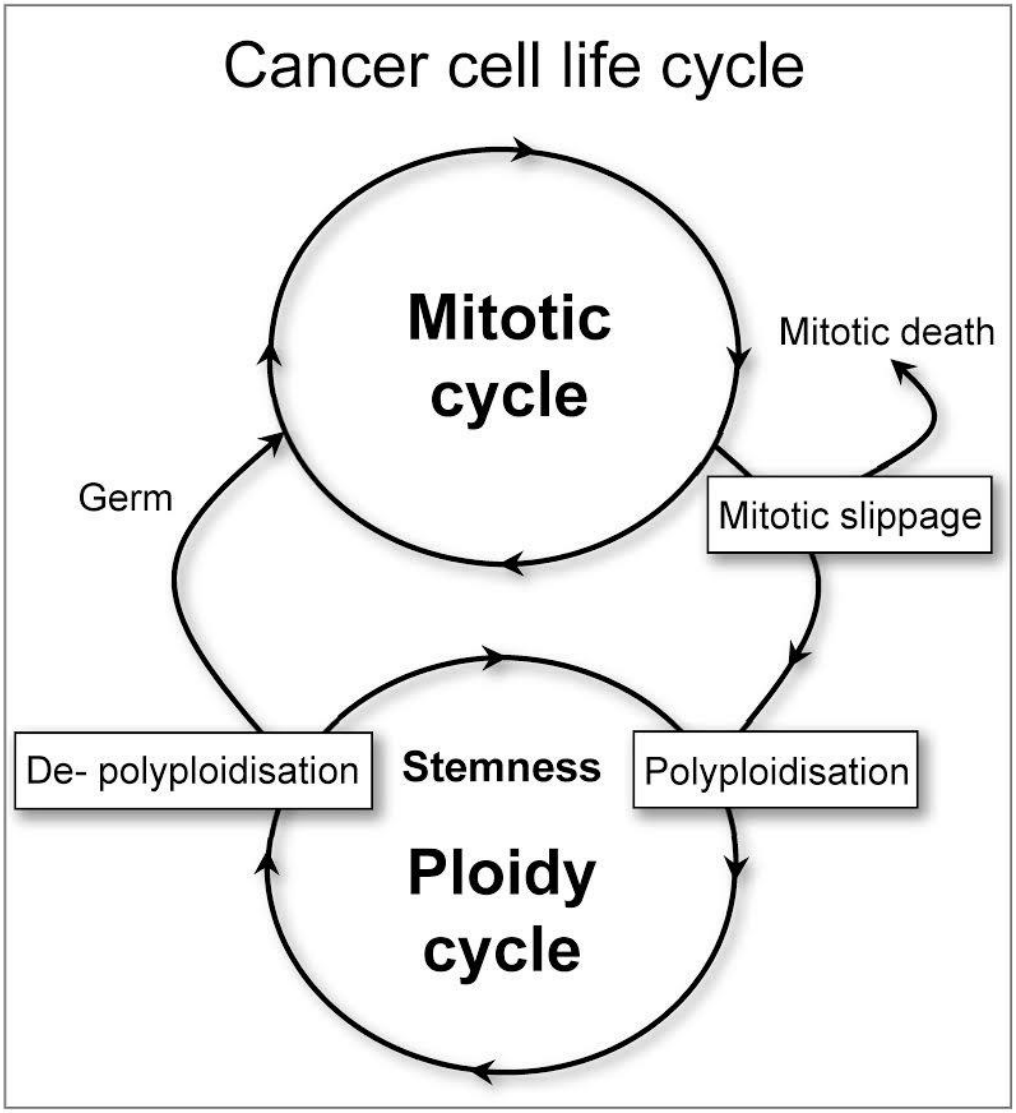

Fig.2. Schematic of the asexual cancer cell "life-cycle' adapted from Erenpreisa and Cragg, 2007. Mitotic slippage serves as a gate to the ploidy cycle producing by induced stemness and re-replication followed by de-polyploidisation the totipotent "germ" cell, which replenishes the Hayflick limit for replicative immortality. Both cycles are reciprocally accessed through asymmetric cell divisions

Transition from mitotic cycle to tetraploidy is often occurring by mitotic slippage (Walen 2017). Moreover, it also seems that through this mechanism polyploid cancer cells not only quit the diploid cell cycle but also resurrect the life-cycle of an ancient eukaryote. They become not only de-differentiated but apparently de-speciated. Our preliminary observations (exemplified in

Fig.3) indicate that they can acquire amoeboid motility, encyst and excyst, and support reproductive immortality by a process akin to sporogenesis.

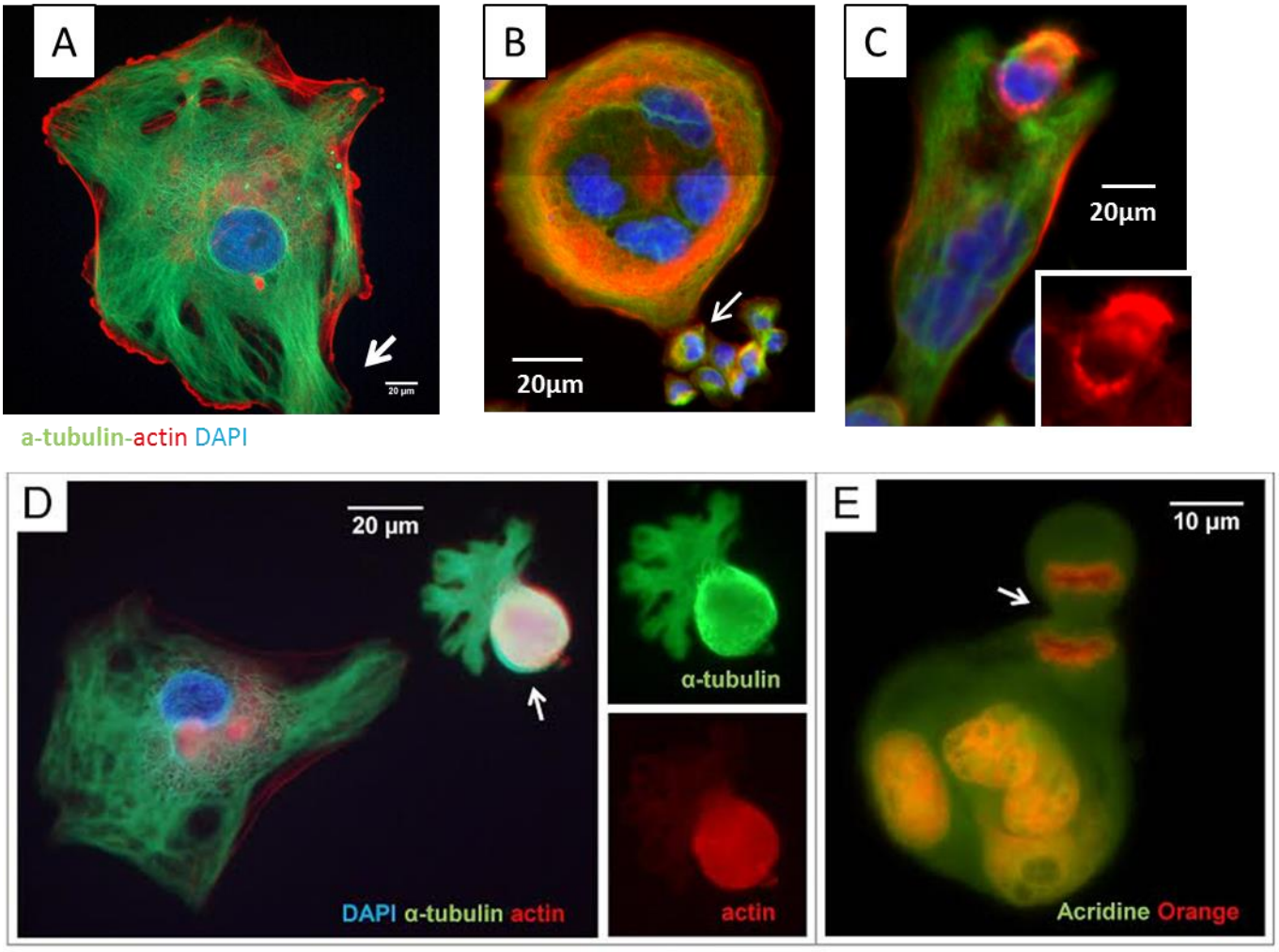

Fig.3. The protozoan features of motion and reproduction of polyploid human cancer cells: (A) Enrichment of cytoskeleton elements; arrowed is the extending pseudopodium of the front movement edge; (B) encystment and excystment by releasing small subcells (spores); (C) budding of a sub-cell trough the actin ring. The apical pole of the bud possesses a typical actin "cap'' (insert); (D) A mobile cell (arrowed) highly enriched in actin and tubulin (inserts); (E) budding of a cellularised sub-nuclei immediately starting symmetric mitosis from a multi-nucleated cell. (A,D) MDA MB 231 cells on day 19 after doxorubicin treatment; (B,C) A431cells cultivated with metformin for one year; (E) non-treated MCF-7 cell in culture.

The budding of treatment resistant small tumour cells was first observed and called “sporosis” by Buikis et al., (1999), and further documented through live-cell imaging as a means of self-renewal, coined “neosis” by Sundaram et al., 2004; Rajaraman et al., 2005. Other authors have also observed similar behaviours (Erenpreisa and Cragg 2008 ; Zhang S et al., 2014; Diaz-Carballo et al., 2014; Zhang D et al., 2014; Niu et al., 2017) which we generalised as a cancer cell “life-cycle” (Erenpreisa and Cragg 2007), comparable with many features occurring in UC organisms for example amoeba (Niculescu 2016). The capability of single polyploid tumour cells induced by stress to produce spheroids and initiate tumours in vivo has now also been shown (Weihua et al., 2011; Zhang S et al., 2014).

Conclusion:

The conceptual framework for the origins of cancer has been debated for centuries. As our technical ability to study cancer cells and compare them to normal cells has developed the level of complexity has increased exponentially. Nonetheless the fundamental concept that cancer in some way accesses earlier developmental states has persisted. Previously this was observed as the concept of de-differentiation from somatic cells to a more stem-like precursor. Here, we go further and propose that this de-differentiation, achieved through a combination of successive epigenetic reprogramming cycles during polyploidisation and non-linear thermodynamics-mediated self-organisation allows the tumour cells to enter transcriptional space of their ancient UC eukaryotic precursors. This descent down the phylogenetic tree admits access to the UC reproduction program supporting immortality that is a fundamental hallmark of cancer from which different treatment approaches may emerge.

Acknowledgement

JE appreciates the fruitful discussions with Professor Vladimir Niculescu. We thank Mr. Jekabs Krigerts for formatting the article.

Conflict of interests None.

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