

Prospects & Overviews

Transmissible cancers in an evolutionary context

Beata Ujvari^{1) 6)*}, Anthony T. Papenfuss^{2) 3) 4) 5)} and Katherine Belov⁶⁾

Cancer is an evolutionary and ecological process in which complex interactions between tumour cells and their environment share many similarities with organismal evolution. Tumour cells with highest adaptive potential have a selective advantage over less fit cells. Naturally occurring transmissible cancers provide an ideal model system for investigating the evolutionary arms race between cancer cells and their surrounding micro-environment and macro-environment. However, the evolutionary landscapes in which contagious cancers reside have not been subjected to comprehensive investigation. Here, we provide a multifocal analysis of transmissible tumour progression and discuss the selection forces that shape it. We demonstrate that transmissible cancers adapt to both their micro-environment and macro-environment, and evolutionary theories applied to organisms are also relevant to these unique diseases. The three naturally occurring transmissible cancers, canine transmissible venereal tumour (CTVT) and Tasmanian devil facial tumour disease (DFTD) and the recently discovered clam leukaemia, exhibit different evolutionary phases: (i) CTVT, the oldest naturally occurring cell line is remarkably stable; (ii) DFTD exhibits the signs of stepwise cancer evolution; and (iii) clam leukaemia shows genetic instability. While all three contagious cancers carry the signature of ongoing and fairly recent adaptations to selective forces, CTVT appears to have reached an

evolutionary stalemate with its host, while DFTD and the clam leukaemia appear to be still at a more dynamic phase of their evolution. Parallel investigation of contagious cancer genomes and transcriptomes and of their micro-environment and macro-environment could shed light on the selective forces shaping tumour development at different time points: during the progressive phase and at the end-point. A greater understanding of transmissible cancers from an evolutionary ecology perspective will provide novel avenues for the prevention and treatment of both contagious and non-communicable cancers.

Keywords:

■ cancer evolution; canine transmissible venereal tumour; clam leukaemia; micro-environment; Tasmanian devil facial tumour disease.

Transmissible cancers are excellent models to investigate cancer evolution

The evolutionary and ecological framework generally applied to organismal evolution has also been suggested to apply to cellular processes, such as tumorigenesis [1–3]. Similar to abiotic and biotic selection processes imposed on organisms, the cellular micro-environment as well as the host immune system may enforce similar selective regimes on individual cancer cells [1, 2]. Cells that acquire selective advantages via genetic and/or epigenetic modifications are able to proliferate autonomously, avoid immune recognition and undergo clonal expansion. Intra-tumour karyotypic, genomic, epigenomic and transcriptomic heterogeneity provide the evolutionary landscape for individual cancer cells to adapt to selection pressures imposed by the micro-environment and rapidly acquire novel cancer phenotypes, immortalization and/or increased invasiveness and resistance [2, 3]. Contagious cancers, such as canine transmissible venereal tumour (CTVT) and Tasmanian devil facial tumour disease (DFTD), provide unique models for studying cancer evolution because such tumours are not only able to escape immune editing and metastasize to distant organs but also able to afflict new hosts [4] (Fig. 1, Table 1). Several studies have begun to evaluate DFTD and CTVT development and progression from an immunological and evolutionary point of view, using a combination of molecular and cytogenetic technologies [5–16]. Thus far, these studies have focused on investigating the accumulation of genetic and karyotypic alterations as tumours progress, whereas the impact

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¹⁾ Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria 3216, Australia

²⁾ Bioinformatics Division, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia

³⁾ Department of Medical Biology, University of Melbourne, Parkville, Victoria 3010, Australia

⁴⁾ Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria 3010, Australia

⁵⁾ Bioinformatics and Cancer Genomics, Peter MacCallum Cancer Centre, East Melbourne Victoria, 3002, Australia

⁶⁾ Faculty of Veterinary Sciences, University of Sydney, Sydney, New South Wales 2006, Australia

*Corresponding author:

Beata Ujvari

E-mail: beata.ujvari@deakin.edu.au

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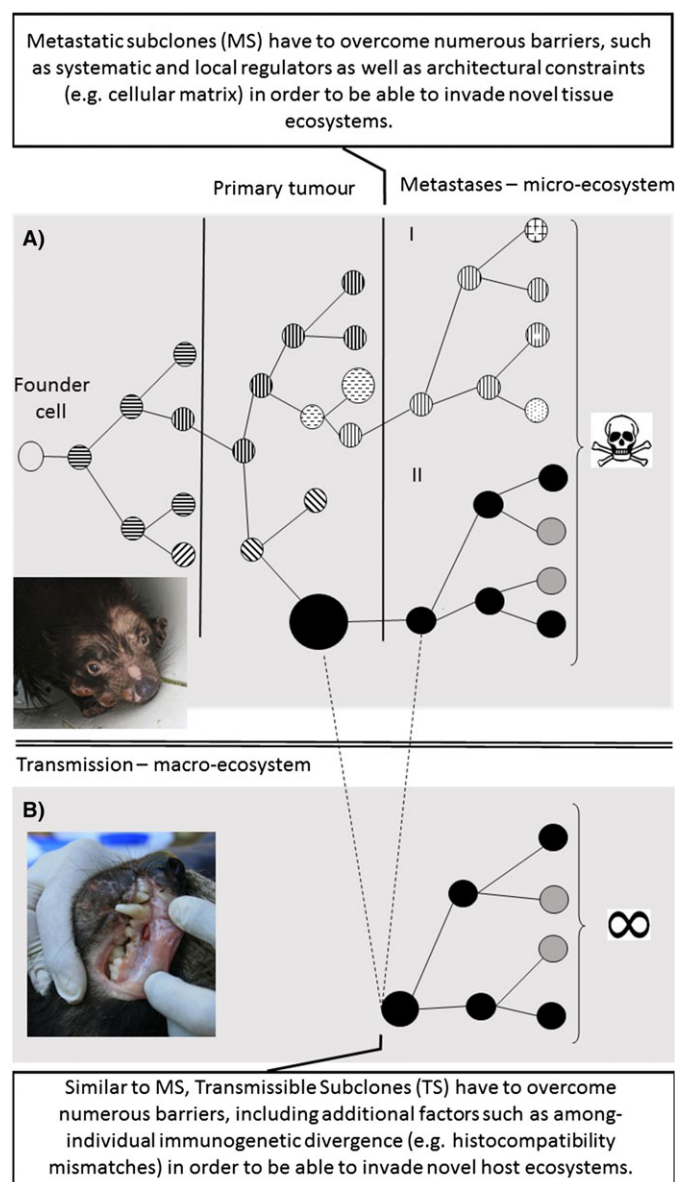


Figure 1. The evolution of non-communicable and transmissible cancers. **A:** The evolution of cancer within the host (modified from Greaves and Maley [19]). Mutant subclones (MS) with higher proliferative potential gain higher fitness and are able to expand, while other subclones become extinct or remain dormant. Vertical lines represent selective pressures within the host (i.e. micro-environmental factors, such as the immune system of the host and competition for resources). Each differently patterned circle represents a genetically distinct subclone, and circle size represents minor or major subclones. Metastases–micro-ecosystem I–II represent local niches (different tissue ecosystems) within the host. MS have to overcome numerous barriers, such as systematic regulators (hormones, growth factors, immune/inflammatory response cells and cytokines), local regulators (oxygen/metabolism/nutrients, cell–cell and cell–stroma/matrix) and architectural constraints (physical compartments and basement membranes) in order to be able to invade novel tissue ecosystems. The “skull and crossbones” indicate the ultimate fate of non-transmissible cancer, that is, cancer cells go extinct with the death of their host. Grey area depicts individual host. **B:** The evolution of transmissible cancer between hosts. Subclones with the capacity to transmit to a novel host gain higher fitness and hence are able to expand, while other subclones become extinct with the death of the progenitor host. Horizontal double lines represent selective pressures between the hosts (i.e. macro-environmental factors, such as the genotypes and the immune system of the individual hosts). Each differently patterned circle represents a genetically distinct subclone, and circle size represents minor or major subclones. Transmission–macro-ecosystem represents an ecological and evolutionary niche across the affected species. As mentioned earlier, transmissible subclones have to overcome similar and additional barriers to MS, such as physical transmission constraints (physical transfer/motility across membranes), local and systematic regulators (oxygen/metabolism/nutrients, cellular matrix, population genetic diversity and ethology of the species, histocompatibility mismatches, respectively) in order to be able to invade novel host ecosystems. Dashed lines depict the uncertainty whether the ancestral cell line in transmissible cancers have originated from a primary or from metastatic tumour. Grey areas represent different host individuals. The “eternity symbol” indicates the ultimate fate of transmissible cancers; they are able to survive even after the death of the host, and therefore, they can become immortal.

Table 1. Comparison of the three naturally occurring transmissible cancers, the CTVT, the DFTD and the clam leukaemia

Disease	CTVT	DFTD	Clam leukaemia
Species affected	Dogs, wolves, coyotes and jackals	Tasmanian devil	Soft-shell clams
Distribution	Worldwide	Tasmania	North Atlantic coast
Appeared	11 000 years ago	>20 years ago	>40 years ago
Emerged owing to	Low genetic diversity of host species	Low genetic diversity of host species	Pollution, temperature and overcrowding
Transmission via	Direct contact during sexual intercourse	Direct contact during social interaction	Filtration of seawater contaminated with neoplastic cells
Primary tumours	Genitalia	Face	Haemolymph
Metastasis	Rare	65%	100% (invade all tissues)
Mortality	Rare	Close to 100%	Close 100%
Spontaneous regression	Within 6–9 months	Rare	No information
Origin	Myeloid cell	Schwann cell	Haemocytes
Potential role of retrotransposons in cancer initiation	Yes (<i>L1NE-1</i>)	No	Yes (<i>Steamer</i>)
Karyotype	Signatures of chromothripsis, but relatively stable	Signatures of chromothripsis, although relatively stable novel strains are being described	Aneuploidy and polyploidy (tetraploids and pentaploids)
Genome	Relatively stable	Relatively stable	Several small microsatellite expansions and deletions showing significant divergence in cancer cell subgroups specific to the USA and Canada clam populations
Telomere homeostasis	No information	Dynamic, shows temporal variation, potential signs of ongoing phenotypic adaptation	No information
Cell-surface markers	Shows inter-tumour and intra-tumour heterogeneity	Shows inter-tumour and intra-tumour heterogeneity	200-kDa cell surface antigen defined by monoclonal antibody 1e10
Epigenetic regulation	Active in regulating cell surface MHC expression	Active in regulating cell surface MHC expression	No information
Immune system modulation	MHC down-regulated during progressive phase, then up-regulated during regressive phase	Shows temporal variation, potential signs of ongoing phenotypic adaptation	Bivalves may utilize a histocompatibility system that may be unique to molluscs or evolutionarily related to Fu/HC or MHC
Evolutionary status	Appears to have reached a stalemate with the host	MHC down-regulated during progressive phase	Shows the signs of stepwise evolutionary phase

CVT, canine transmissible venereal tumour; DFTD, devil facial tumour disease; MHC, major histocompatibility complex; Fu/HC, fusion/histocompatibility.

of micro-environmental changes in tumour evolution has largely been neglected. The recent discovery of a horizontally transmitted clonal leukaemia in soft-shell clams (*Mya arenaria*) [17] demonstrates that transmissible cancers can affect both vertebrates and invertebrates, and most likely are more common in nature than previously expected [17]. In the current review, we focus on the evolution of the two vertebrate cancers, CTVT and DFTD, and only briefly touch on the clam leukaemia.

Transmissible cancers adapt to both their micro-environment and macro-environment

Evolutionary theories applied to organisms are also relevant to cancer

The mechanism underlying cancer evolution has attracted avid interest over the last 50 years, and two mutually non-exclusive, but concurrent, theories have been developed to describe neoplasm progression. (i) The classic model of stepwise carcinogenesis posits that during tumour development, a transformed cell or cells gain unlimited proliferative capacity and hence produces uncontrolled cell growth. The subsequent accumulation of random mutations results in heterogeneous cell subpopulations within the tumour, and the concomitant selection of increasingly malignant subclones drives tumour evolution [1, 18, 19]. (ii) The hierarchical model, or cancer stem cell (CSC) hypothesis [20–23], also traces tumour origins to single mutated cells with unlimited proliferative potential, but in contrast to the clonal model, the cells possess stem cell qualities [24]. The concept of CSCs assumes that the development of the tumour results from the clonal evolution of the original CSC population [25, 26]. Although the cancer progenitor cells are thought to be different according to the two theories, that is, somatic cells and CSCs, respectively, both theories portray cancer progression as the accumulation of genetic modifications (mutations and epigenetic alterations) and selection for, and subsequent expansion of, clones with highest survival and reproductive (proliferative) advantage. Interestingly, although transmissible animal cancers have been proposed to provide excellent model systems of the CSC process [27], a preliminary study focusing on the expression profiles of embryonic stem cell and pluripotent germ cell specific genes could not confirm the existence of CSCs in devil facial tumours [28]. However, to univocally confirm or exclude the role of CSCs in transmissible cancer progression, additional experiments must be conducted, including identification of (i) a population of potential CSCs from fresh tumour samples and/or primary cell cultures and (ii) sarcosphere formation and self-renewal assays, followed by gene signature analyses of stem cell markers, and (iii) once a possible stem cell population has been identified, the evaluation of number of cells required from the enriched CSCs and from primary cancers to initiate a tumour when xenotransplanted into immuno-compromised mice would be the ultimate proof of CSC existence [28].

The general concept has been that cancer cells accumulate genomic alterations in a gradual stepwise manner in order to acquire such selective advantages [29]. However, recent karyotype analyses of cancer cells have suggested a stochastic

cancer evolution model, where the progression of cancer cells alternate between punctuated and stepwise phases [3, 30]. The punctuated phase is characterized by rapid, stochastic karyotype changes resulting in extensive intra-tumour cell heterogeneity. Once punctuated selection has produced a cancer genome with a selective advantage, the subsequent clonal expansion of cancer cells enters a stepwise phase. The initial evolutionary process can involve large-scale chromosomal changes (e.g. chromothripsis), followed by minor alterations resulting in more homogenous cancer cell population. The punctuated phase is characterized by extensive transcriptome alterations in contrast to the stepwise phase when the transcriptome only experiences limited modifications. The progression of cancer genomes (cells) in this model therefore appears to agree with both Eldredge and Gould's "punctuated equilibrium" theory [31] and Richard Dawkins's remark: "Darwin's *survival of the fittest* is really a special case of a more general law of *survival of the stable*" [32]. Although cancers appear to "want to evolve" [33], they actually simply follow the "willy-nilly" of evolution [32] to reach a state of equilibrium [31].

Canine transmissible venereal tumour, the oldest naturally occurring cell line, is remarkably stable

Contagious cancers, CTVT and DFTD, provide interesting models to investigate the evolutionary processes involved in tumour development. The two diseases share similar aetiology; both originated from an aberrant cell line with unlimited proliferative potential and can be transplanted as allografts between unrelated hosts by physical transfer (Table 1). A common characteristic feature of CTVT and DFTD is that both genomic and karyotypic stability is largely maintained upon transmission to a novel host [7, 10, 16]. CTVT is a globally distributed sexually transmitted tumour of dogs (*Canis familiaris*). The disease arose about 11000 years ago in wolves [13] or in one of the ancient breeds of dog [16], which most likely had low genetic variation, and therefore, CTVT is considered to be the oldest known somatic cell line [13, 16]. Despite spreading across continents about 500 years ago and carrying thousands of genomic rearrangements and a massive mutation burden, CTVT is remarkably stable and lacks subclonal heterogeneity [16].

Devil facial tumour disease exhibits the signs of stepwise cancer evolution

Devil facial tumour disease is a more recently emerged contagious disease affecting Tasmanian devils (*Sarcophilus harrisii*) and was first observed in north-eastern Tasmania, Australia, in 1996 [34]. DFTD is passed between devils by biting during social interactions [9]. Molecular genetic studies have confirmed the clonal nature of DFTD [7, 35, 36], and transcriptome analysis has pinpointed the genesis of the disease to peripheral nervous system cells, either Schwann cells or Schwann cell precursors [8]. The cancer causes large ulcerating tumours primarily around the face and jaws of the devils and frequently results in death within 6 months after the emergence of the first lesions [37]. Recent karyotype analyses and genome sequencing of devil facial tumours suggest that the tumour originated in a single female devil [6, 7]. While the clonal nature of DFTD is unequivocal, three recent studies have described the

existence of four, closely related, but karyotypically distinct DFTD strains [6, 7, 10]. The importance of these chromosomal variants for tumour phenotype is not clear, although preliminary cell culture results suggest that the different strains have different growth rates and morphologies [10]. The observed chromosomal rearrangements have been proposed to provide some adaptive advantage to the different tumour variants [6], and distinct strains could result in the emergence of a longer disease progression, reduced lethality and increased immune evasion [10]. Alternatively, distinct strains may be non-adaptive, and simply the result of tumours becoming less stable [10], or they may be reflective of evolutionary breakpoints that are frequently rearranged in the marsupial lineage [6]. Interestingly, despite the existence of chromosomal strains, no significant genomic [7] or epigenomic [11] differences have been observed among the four DFTD strains. However, genomic [7], epigenomic [11], telomere homeostasis [12] and ploidy assays [38] revealed not only high inter-tumour variations but also temporal genotypic and phenotypic DFTD changes. Moreover, we have recently demonstrated that DFTD is able to rapidly respond to novel selective regimes by increasing its number of chromosomes. The presence of aberrant karyotypes in malignant cells is a common feature of both transmissible and non-communicable cancers [6, 17, 39, 40] and have been proposed to be the result of adaptation to the asexual lifestyle of cancer cells. Similar to other asexual organism, cancer cells do not undergo meiotic recombination and therefore are particularly exposed to loss of heterozygosity and the emergence of recessive mutations [39]. By altering normal cell cycles and apoptotic responses, and increasing the tolerance to deleterious mutations, chromosomal rearrangements have been singled out as the counter mechanisms of cancer cells to overcome the detrimental effects of genomic decay [39]. Indeed, elevated tetraploidy in the oldest strain of DFTD indicates that this malignant cell line has once conquered genomic decay by increasing its chromosome numbers. However, there is a trade-off in carrying larger karyotypes, with the concomitant cost of slower growth, and hence most likely explaining why younger strains of DFTD reverted to diploid karyotypes (with the advantage of faster growth and higher proliferative potential). Nonetheless, as we have described earlier, DFTD cells are able to rapidly switch back to tetraploid karyotypes when selection favours slower growing tumours. These results clearly demonstrate that DFTD is a dynamically evolving obligate parasite, with the potential to adapt to the ever-changing evolutionary landscape sculptured by the devils' micro-ecosystem and macro-ecosystem.

Clam leukaemia exhibits genetic instability

The recent discovery of the contagious clam leukaemia shows that transmissible cancers can also affect invertebrates and might be more widespread in nature, particularly in the marine environment, than previously thought [17]. Disseminated, haematopoietic or hemic neoplasia, characterized by abnormal amplification of cells in the haemolymph, occurs in many bivalves (clams, oysters and cockles) and have been suspected to be caused by viral infections [41, 42]. However, the elegant study of Metzger et al. [17] proved that this leukaemia-like cancer of soft-shell clams has most likely been derived from a single original clam and is spreading between animals as a

horizontally transmissible clonal cell lineage. The origins of the disease have been linked to the activation of the retroelement *Streamer* [43], which might have been initiated by environmental stressors, such as pollution, temperature and overcrowding [42, 43]. The disease was first observed in the 1970s and since has spread along the east coast of North America, most likely via filtration of seawater contaminated with neoplastic cells, and caused the decimation of soft-shell clam populations [17] (Table 1). The diseased cells lose their phagocytic abilities, express a novel surface antigen defined by the monoclonal antibody 1e10 and display cytoplasmic sequestration of the p53 tumour suppressor by mortalin tethering [44]. Arriagada et al. [43] proposed that the activation of *Steamer* in *M. arenaria* bears the signatures of a catastrophic genomic instability, which could have contributed to the initiation and development of clam leukaemia. Furthermore, the neoplastic clam cells are characterized by higher than normal DNA content and aneuploid and/or tetraploid karyotypes [45], indicating that the cells most likely have undergone major chromosomal rearrangements. Significant genetic divergence, characterized by several microsatellite expansions and deletions and the appearance of at least one mtDNA single-nucleotide polymorphism, has been observed between cancer cell subgroups across Canada and the USA [17]. In summary, clam leukaemia shows the signature of punctuated (*Steamer* activation and genomic instability) and stepwise (emergence of genetically different subclones) cancer evolution phases. The increased chromosomal numbers observed in the neoplastic haemocytes potentially are either indicative of the initiating catastrophic chromosomal rearrangement or show the signs of adaptation to and overcoming genomic decay as observed in DFTD. Additionally, the unique transmission of these cancer cells, survival in natural seawater and being transmitted via filtration [46], demonstrates their high adaptability to both the hosts' micro-environment and macro-environment, although further studies are clearly necessary to investigate the evolution of this fascinating invertebrate cancer. The observed genetic, chromosomal and phenotypic characteristics clearly indicate that various selective forces (e.g. pollution, overcrowding and hosts' micro-environment) are actively shaping the development and progression of these neoplastic cells.

Canine transmissible venereal tumour and devil facial tumour disease exhibit different evolutionary phases

The gross karyotype rearrangements of both cancers, as demonstrated by chromosomal painting in DFTD [6] and microarray-based comparative genomic hybridization analyses in CTVT [47], raises the possibility that these contagious tumours may have been initiated in a single episode of chromothripsis [6, 30]. Although one would expect that disruption of chromosomal integrity will be detrimental to the affected cells and hence will be negatively selected, recent studies have shown that rare catastrophes can actually promote cellular transformation and confer significant selective advantage to the clone, hence concomitantly promoting the evolution toward cancer [30, 48]. According to Stephens et al. [30], such one-off cellular catastrophe appears to have occurred in 2–3% of all human cancers and may be particularly frequent in bone cancers.

The persistence of DFTDs and CTVTs is unique, as these cancer cell lines have survived long after the death of the original hosts and hence most likely have experienced significant opportunities for evolutionary change. The high chromosomal stability of CTVT cells indicates that this cell lineage has well adapted to its niche [16] and most likely has reached the final stages of stepwise evolution.

In contrast, the presence of DFTD strains is puzzling: although this contagious cell lineage seems to have reached evolutionary equilibrium (i.e. genomic stability), the existence of the four karyotype strains may represent different adaptive trajectories of the original clonal cell line. We propose that if DFTD strains have evolved as a result of different adaptive trajectories, their chromosomal patterns could reflect divergence in their overall gene expression profiles. Therefore, further studies should focus on investigating whether the karyotypic divergence observed in DFTD strains results in concomitant differences in transcriptome profiles.

Tumour micro-environment shapes the evolution of transmissible cancers

An optimal niche provided by stromal cells is necessary to tumour progression

Numerous studies have demonstrated that the tumour micro-environment is not a mere bystander of neoplasia but rather actively contributes to tumour initiation and supports carcinogenesis, progression and metastasis [49]. In non-transmissible cancers, the communication between cancer cells and their micro-environment has been described as bidirectional: tumour cells adapt to circumvent the normalizing cues of the micro-environment, and in turn, the micro-environment evolves to accommodate the malignant cells [49]. Tumour–stromal cell interactions are driven by dynamic paracrine and autocrine signalling as tumours evolve, resulting in gene expression changes in both stromal and tumour epithelial cells [50–54]. A still unanswered question is the evolutionary background for metastatic progression: would clonally selected abnormal stromal cells (“fellow-travellers”), which provide an optimal niche for primary tumour cells, accompany metastatic cells during the course of metastasis? Or would novel genetically abnormal (but tumour accommodating) stromal environments arise at each site of tumour metastasis? Transmissible cancers could potentially harbour the answers to these questions. Not only have CTVT and DFTD been shown to metastasize to distant organs [4, 37], but also their transmission from animal to animal can be described as an inter-individual metastasis. With every transmission, these malignant cell lineages have to overcome the challenges of the novel micro-environment and establish an accommodating niche, not only within the same organism but also across individuals. If stromal cells are transmitted together with tumour cells, then the cells (progenitors of fellow-travellers) surrounding these transmissible tumours should reflect the stromal niche of nascent CTVT and DFTD cells at their time point of initiation 11 000 and 20 years ago, respectively. A “tumour-welcoming” stromal environment arising at each site of transmission might actually have contributed to the success of these transmissible cancers that have affected

>100 000 dogs and devils. However, co-transmission may also present an additional immune target via the stromal cells, which would not be protected by the currently understood mechanism, for example, cancer cells avoiding immune recognition by down-regulating cell-surface signalling molecules (more details provided later) [55]. Successful co-transmission of cancer and stromal cells suggests that similar to the malignant cells, the fellow-travellers also escape the hosts' immune system via a so-far-unknown mechanism. Additionally, the observation that clam leukaemia cells are being able to survive in natural seawater conditions >6 h [46] poses the question whether fellow-travellers, if they exist, would have a role in facilitating adaptation to and survival in various macro-environmental conditions. Whether transmissible cancer cells have evolved to be self-sufficient, continuing to grow and spread without the need of supporting stromal cells, or exploit the benefits of the optimal niche created by their fellow-travellers, remains a question. In-depth investigations of tumour and associated stromal cells' genomes, transcriptomes and epigenomes in various stages of tumour progression and transmission would be necessary to address and answer these questions. Combined analyses of cancer cells and non-host derived stromal cells within CTVT and DFTD tumours (including both primary and metastatic tumours) could provide optimal avenues to confirm or disprove the existence of fellow-travellers. Studies conducted on transmissible cancers will also have implications for human malignancies. The existence of fellow-travellers in human cancers remain to be proven, but based on the low frequency of circulating tumour cells ($<10^{-8}$) detected in patients with advanced stage tumours, the probability of tumours evolving to be self-sufficient and continuing to grow without the need of supporting stromal cells at the site of metastasis appears to be extremely low [49].

Furthermore, mathematical modelling of cancer invasion has demonstrated that harsh tumour micro-environment conditions (e.g. hypoxia and heterogeneous extracellular matrix) exert dramatic selective forces on the tumour and select for aggressive phenotypes [56]. In contrast, mild conditions select for tumours with smooth, non-invasive margins with a heterogeneous tumour mass containing clones with less and more aggressive traits. The latter scenario may suggest that the first transmission of progenitor CTVT, DFTD and clam leukaemia tumours to the hostile micro-environment of the first vertebrate and invertebrate recipients has contributed to the development of “super-clones” with exceptionally aggressive and invasive phenotypes.

Canine transmissible venereal tumour serves as the model of metabolic mutiny

A common property of cancer cells is up-regulation of glycolysis even in the presence of oxygen, resulting in increased glucose consumption. Aerobic glycolysis, or fermentation of glucose to lactic acid in the presence of oxygen, has been coined the “Warburg effect” after the first observations of Otto Warburg in the 1920s [57]. As aerobic glycolysis is less efficient than oxidative phosphorylation for generating ATP, the role of Warburg effect in cancer development and progression has been a long-standing enigma [58, 59]. Several explanations have been proposed [59–61]: (i) the high-energy view

proposing that aerobic glycolysis enables rapidly dividing tumour cells to harness additional ATP while also generating essential biosynthetic building blocks (nucleic acid, amino acids, lipids, etc.) for their proliferation and (ii) the persistent metabolism of glucose to lactate even in aerobic conditions is a defence adaptation protecting cancer cells from the higher-than-usual oxidative and acid-induced toxic micro-environment [62]. However, studies by Whitaker-Menezes et al. [63] and Ertel et al. [64] suggest a novel explanation by showing that actually oxidative cancer cells nest within a glycolytic stroma, that is, “epithelial cancer cells use oxidative mitochondrial metabolism, while the tumour stromal cells are largely glycolytic by comparison” [64]. The authors used cyclooxygenase-activity staining (technique detecting active mitochondria) and laser-captured compartment-specific profiling data from breast cancer patients to specifically identify the two tumour compartments [63, 64]. These studies put forward the idea of cancer being a rebellion against host ageing, when cancer cells increase their capacity for oxidative mitochondrial metabolism to overcome the detrimental effects of ageing: an overall shift in the entire body toward glycolytic metabolism and/or aerobic glycolysis (resulting from increased oxidative stress and reactive oxygen species and accumulated mitochondrial dysfunction) [64]. As a consequence, the ageing host cells and cancer cells engage in a parasite–host relationship, where cancer cells upregulate mitochondrial oxidative metabolism to exploit the nutrient-rich environment created by the systematic aerobic glycolysis of stromal cells. CTVT has been suggested to be the ultimate example of such metabolic mutiny by cancer cells [64]. Notably, a key factor in the immortality of CTVT is its ability to capture mtDNA from its host, consequentially escaping genomic decay owing to high mutation rates and relaxed selection and allowing for mitochondrial amplification and rejuvenation (a phenomenon not yet observed in DFTD) [7, 14, 64]. Although studies have shown that mesenchymal stem cells and fibroblasts can also transfer intact mitochondria to epithelial cancer cells [65] and concomitantly increase oxidative mitochondrial metabolism, this area of cancer–host interaction has so far received little attention [64]. Transferring mitochondria from the host, or simply amplifying oxidative mitochondrial metabolism in cancer cells, results in two-compartment tumour metabolism, where cancer cells perform oxidative phosphorylation and the stromal cells in the tumour are in glycolysis [64]. Although increased ploidy has been observed in DFTD as a countermeasure of genomic decay [38], in contrast to CTVT, mitochondrial capture has not yet been detected in the devil cancer [7]. Nevertheless, CTVT could serve as a model to investigate and to better understand the two compartments of tumour metabolism (characterized by glycolytic/oxidative metabolic coupling), and to develop drugs specifically targeting this parasitic form of energy transfer [64, 65].

Tumour macro-environment shapes the evolution of transmissible cancers

Although the topic of genetic alterations in tumour-associated stroma is still controversial, genome-wide association studies have unequivocally demonstrated the importance of the host's

genotype in carcinogenesis and tumour progression [49]. Large human cohort and laboratory animal (mice) studies have discovered genotype-specific differences in individual capacity to support tumour growth (via angiogenesis), susceptibility to carcinogenesis and risk of metastatic spread [66–68]. For example, pro-inflammatory cytokine haplotypes have been linked to adverse prognosis in gastro-oesophageal cancer [69], and a point mutation of the nitric oxide-related genetic factor has been associated to advanced disease and bone metastasis in prostate cancer [70]. A human population-based study demonstrated that ethnic background, being of African American origin, increased the risk of developing a rare breast cancer subtype (basal-like breast tumours) in premenopausal women [67]. Interestingly, recent studies demonstrated that different clam populations are more or less susceptible to induction of cancer by DNA damaging agents, hybrids of *Mercenaria* spp. particularly showing high genetic predisposition for gonadal neoplasia [42].

Vertebrate transmissible cancers are believed to have emerged and spread as a result of genetic bottlenecks and low immune gene repertoire diversity in canine and devil populations, particularly at the major histocompatibility complex (MHC) locus [13, 71]. MHC molecules are generally highly polymorphic and play an important role in self-/non-self-recognition, graft rejection and the detection of altered and malignant cells. Previous studies by Siddle et al. [72, 73] found that lack of MHC gene diversity in inbred devils contributed to the rapid spread of DFTD across Tasmania. This led to the proposal that sharing of functionally identical MHC genes by devils and DFTD cells caused the failure of infected devils to recognize the tumour cells as non-self and mount an effective immune response against them. However, recent spread of DFTD to areas with genetically disparate devil populations has revealed that DFTD successfully crosses histocompatibility barriers that would normally prevent allograft acceptance [74]. More recently, a study showed that MHC polymorphism does not affect the spread of the tumour [75]. Rather, DFTD is actively evading the host immune system by down-regulating genes involved in the antigen-processing pathways, resulting in the concomitant loss of cell-surface expression of MHC class I molecules and escape of immune recognition [75].

The canine disease is also believed to have emerged in inbred wolf/dog populations with low MHC diversity and then spread to MHC-disparate hosts when the tumour evolved the ability to evade the host immune response [71, 76]. Following transmission, the immune system fails to control CTVT growth owing to lack of MHC expression on tumour cell surfaces. However, shortly after the progression stage, cell-surface MHC gene expression significantly increases via epigenetic modifications; concomitantly, the immune system recognizes the malignant cells, and the tumour either stabilizes or regresses [74, 77]. While regressed CTVT tumours maintain the potential of transmission to novel hosts, in contrast to DFTD, CTVT rarely causes the death of the host. CTVT is clearly an ancient cell lineage that, over 10000 years, has acquired adaptive strategies to reach a dynamic stalemate within the host's micro-environment. In contrast, DFTD appears to be driving itself and its host to extinction by rapidly killing the infected devils [74]. Recent studies have shown that DFTD uses various pathways, including gene expression alterations [11, 12], telomere homeostasis [12], epigenetic variations [11] and increased ploidy [38] to adapt to the ever-

changing evolutionary landscape sculptured by the devils' micro-environment. Compared with CTVT, DFTD is a recently emerged cell lineage, and hence, it is questionable whether the tumour clones and their host micro-environment would have had time to reach a co-evolutionary equilibrium.

Interestingly, although molluscs and other invertebrates do not possess MHC, it has been proposed that they may employ other self-/non-self-recognition mechanisms (a system that may be unique to molluscs or evolutionarily related to the fusion/histocompatibility system of colonial ascidians or to MHC) to recognize and combat transformed malignant cells [17, 78]. Whether these histocompatibility systems are genetically and/or epigenetically modulated in clam leukaemia remains to be answered.

Apart from MHC, the analyses of other cell-surface markers (e.g. lysozyme-40, vimentin, desmin, nestin, etc. [8, 27, 79–82]) have revealed that despite the clonal nature of vertebrate transmissible cancers, there is significant heterogeneity of lineage marker expression both within the tumour cell population and between individual tumours (reviewed by O'Neill [27]). The observed high intra-tumour and inter-tumour phenotypic heterogeneity on stable genomic backgrounds demonstrates that tumour cells engage in an incessant crosstalk between each other and their micro-environment and phenotypically adjust to the changes of the surrounding stroma.

Concluding remarks and future perspectives

The two naturally occurring vertebrate transmissible cancers represent two evolutionary scenarios and timeframes of tumour evolution. CTVT has co-evolved with its canine hosts for over 10 000 years and appears to have reached an evolutionary equilibrium in its micro-environment (within host) and macro-environment (between hosts). In contrast, DFTD emerged about 20 years ago, and hence, this cancer still shows the signs of a stepwise evolution characterized by rapid but minor phenotypic and genotypic alterations. The invertebrate cancer potentially represents an intermediate step, it has been present for over 40 years and distinct subgroups have emerged across its distribution. It appears that all three contagious cancers might have originated from a one-off event of chromosomal shattering (punctuated phase of cancer evolution). Once clones with highest proliferative, immune evasion and transmissibility potential gained the highest fitness and were selected for, the three malignant cell lines have most likely entered the stepwise phase of their evolution. While all three contagious cancers carry the signature of ongoing and fairly recent adaptations to their micro-environment and macro-environment (e.g. mtDNA capture in CTVT, increased ploidy in DFTD, microsatellite subclones in clam leukaemia, and phenotypic inter-tumour and intra-tumour heterogeneity in both CTVT and DFTD), CTVT appears to have reached an evolutionary stalemate with its host, while DFTD and the clam leukaemia appear to be still at a more dynamic phase of their evolution. The parallel investigation of genome and transcriptome profiles of contagious tumours and their immediate micro-environment could shed light on the selective forces shaping tumour development at different time

points: during the progressive phase and at the endpoint. By identifying and distinguishing between the evolutionary processes influencing tumour growth and transmission at specific cancer life-history stages, it could be possible to facilitate the development of focused and pertinent therapies. For example, although many tumours evade host immune surveillance by down-regulating cell-surface MHC expression, the scarcity of *in vivo* models has led to slower progress on immunological aspect of cancer research [77]. Furthermore, because cancer involves complex evolutionary processes, therapies commonly used today are evolutionary “myopic”, killing cells on one hand but on the other hand also selecting for cells that are resistant to the therapy, concomitantly resulting in drug resistance [83]. Using CTVT as an *in vivo* model and understanding the mechanisms underlying the homeostasis reached between CTVT growth and the host immune system [55] could fill this gap and provide alternative avenues to control tumour growth and to reduce acquired therapeutic resistance [77, 83]. Unlike CTVT, DFTD and clam leukaemia are younger contagious cancers that have not reached an evolutionary balance with their hosts' ecosystem. A comparison of the evolutionarily stable CTVT with DFTD in evolutionary flux could highlight the currently imperceptible genetic and/or epigenetic pathways and mechanisms that make the difference between a relatively benign tumour and a fatal cancer. Identification of the key mechanisms keeping malignant cells under control (without killing them and concomitantly selecting for more aggressive, drug-resistant clones) could provide novel intervention points and strategies for treating both human and animal cancers.

Moreover, therapies targeting micro-environmental conditions surrounding the tumour cells could reduce aggressive (contagious and metastatic) tumours by decreasing the impact of extreme selective forces. As we have mentioned earlier, CTVT could serve as the perfect model to study mitochondrial transfer and metabolic interaction between the cancer and neighbouring stromal cells, an area so far somewhat overlooked in human cancer research [64]. Blocking mitochondrial transfer from the host, or disrupting the balance between the two compartments of tumour metabolism (characterized by glycolytic/oxidative metabolic coupling) by targeting the evolutionary stable stromal cells, instead of the more volatile cancer cells, could provide future avenues for cancer therapies [64, 65].

Apart from the immediate micro-environment of cancer cells, the host's macro-environment (e.g. host's genotype) is also a key selective force in the evolutionary arms race between cancers and their hosts (Fig. 1). Therefore, establishment of a genetic profile for each patient, or animal in the case of contagious cancers, may provide crucial information concerning the prediction of disease outcome, and for personalized treatment.

All three transmissible cancers are actively interacting with and adapting to their micro-environment and macro-environment and showing the signs of ongoing evolutionary arms race between these unique malignant parasites and their hosts. The importance of this evolutionary struggle should not be overlooked when developing control strategies, including vaccines, immunotherapies or other interventions, to halt the progression of both human and animal cancers. A recent study by Kreiss et al. [84] showed that immunizing Tasmanian devils with killed DFTD cell preparations coupled with adjuvants has induced humoral and cytotoxic immune responses against

DFTD cells in the devils. However, the protection was short term, and the re-challenge (with the same and also different strains of DFTD) 1 year later resulted in tumour growth [84]. As previously demonstrated, DFTD can rapidly adapt to anthropogenic selection via altering chromosomal numbers, providing a cautionary example that novel chromosomal DFTD strains or phenotypic variants might be able to counter-evolve and outrun the developed vaccine, or other immunotherapies.

In general, cancer progression is a dynamic process; therefore, treatments addressing the evolutionary stages of tumour development could provide an alternative, and potentially more efficient, solution compared with the currently used “one fits all” approaches. In summary, transmissible cancers can teach us about the co-evolution of cancer cells and their environment and may have the potential to inform alternative preventive and therapeutic strategies in both animal and human cancers.

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