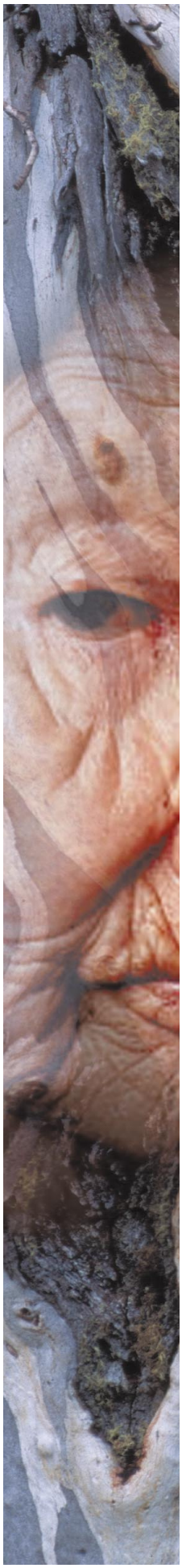

The age of cancer

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A striking link exists between advanced age and increased incidence of cancer. Here I review how several of the age-related molecular and physiological changes might act in concert to promote cancer, and in particular epithelial carcinogenesis. Experimental data indicate that the aged, cancer-prone phenotype might represent the combined pathogenetic effects of mutation load, epigenetic regulation, telomere dysfunction and altered stromal milieu. Further verification of the role of these effects should in turn lead to the design of effective therapeutics for the treatment and prevention of cancer in the aged.

Advancing age is the most potent of all carcinogens. In humans, the incidence of cancer rises exponentially in the final decades of life, culminating in a lifetime risk of 1 in 2 for men and 1 in 3 for women[1]. This dramatic age-dependent escalation in cancer risk is fuelled largely by a marked increase in epithelial carcinomas from ages 40 to 80 years, as opposed to cancers of mesenchymal or haematopoietic origin (Fig. 1). What underlies this intimate link between cancer and advanced age? At first glance, one may presume that the sequential accumulation of somatic mutations over a lifetime finally drives a subset of cells in the organism over a critical threshold, leading to emergence of cancers later in life. If so, then why do epithelial carcinomas predominate? Even more perplexing is the fact that most laboratory mouse strains approaching a biologically equivalent age succumb instead to mesenchymal and haematopoietic malignancies. Here I consider the thesis that a number of molecular and physiological processes underlie the rate and patterns of cancer in the aged. Specifically, I propose that the cancer-prone phenotype of older humans might reflect the combined effects of cumulative mutational load, increased epigenetic gene silencing, telomere dysfunction and altered stromal milieu.

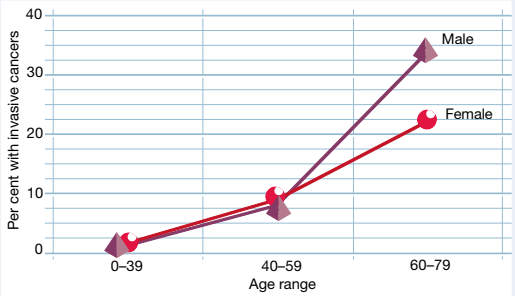

Figure 1 Cancer incidence as a function of age. Incidence of invasive cancer plotted against age ranges reveals exponential increase from age 40–80 years (ref. 1). Purple, male; red, female. Note that beyond age 80, incidence of cancers plateau[94].

Genome maintenance in ageing mice and humans

Mutation rates and cancer

Cancer is clearly a disease of the genes[2]. Tumour progression is driven by clonal selection and evolution of tumour cell populations[3], a model well substantiated by serial mutational analyses of staged human colorectal cancers[4] as well as other cancer types[5–8]. An increase in somatic mutations has been documented in aged cells and tissues of both humans and mice, and presumably relates to cumulative lifetime exposure to endogenous and exogenous DNA damaging agents[9,10], as well as to an accumulation of mutations resulting from proof-reading and mismatch errors during DNA replication[11]. Less clear is whether this process is exacerbated by a progressive decline in DNA monitoring and repair capability. The fundamental question remains whether the rates of spontaneous mutation *in vivo* are of sufficient magnitude to initiate and drive the transformation process and ultimately generate the wholesale genomic alterations encountered in adult human cancers. As a corollary, one wonders whether mutational rates differ sufficiently among various tissues to provide a basis for the observed tumour spectrum of later life.

It has been estimated that, in cultured human cells, the spontaneous mutation rate is approximately 2×10^{-7} per gene per cell division[12]. If this rate remains fixed over a lifetime and represents an accurate measure of *in vivo* mutational events across the intact organism, then each cell would accumulate only a few mutations over a lifetime[13]. This rate would be well below the predicted value of between four and ten rate-limiting, stochastic events for tumour initiation and progression, which was extrapolated from cancer trends in ageing populations and from transgenic models of tumorigenesis[14–16]. The observation that rates of spontaneous mutations seem insufficient to account for the extensive tumour-associated genomic changes led to the concept, and subsequent proof, of the 'mutator phenotype'[17,18]. In this scenario, the age-dependent increase in cancer stems from the eventual mutation of genes governing genome stability, mutational inactivation of which would lead to an accelerated pace of mutations overall[17]. The spontaneous loss of function, or perhaps haploinsufficiency, in these genome maintenance pathways might also provide a basis for the well-recognized inherent instability of tumour cell genomes relative to their normal counterparts[19,20]. Perhaps the disconnect between mutation rates and cancer genome profiles can be explained in part by the clonal expansion of cancerous stem cells harbouring initiating lesions. This basic idea is a reasonable one, although there is evidence that robust checkpoint mechanisms could prevent further expansion of nascent cancer cells harbouring oncogenic lesions, for example, Ras-induced senescence or Myc-induced apoptosis[21]. Thus, although mutator and clonal expansion mechanisms are likely to contribute to the

increase in cancer as a function of age, these mechanisms have not yet been documented in the context of ageing tissues and fail to provide a unifying principle that accounts for the tissue distribution and cytogenetic profiles of most adult cancers.

An alternative view proposes that mutation rates calculated on the basis of biological age rather than number of cell divisions could more closely approximate the requisite number of mutations presumed necessary for initiation of tumorigenesis[22]. Such rates are in accord with those empirically determined in mice harbouring an integrated mutational reporter[23,24]. These model systems, reviewed by Vijg and Dolle[25], have revealed tissue-specific differences in mutational rates (for example, intestine and liver > heart > brain) as well as radically different mutational spectra in these aged tissues, the latter pointing to organ-specific differences in genome maintenance mechanisms. However, these studies indicate that age-dependent rates of mutation do not correlate strictly with rates of cell proliferation nor do they readily explain the observed tumour spectra of aged individuals.

Age-dependent deterioration in genome maintenance

Hereditary cancer and progeric syndromes (the latter characterized by physical symptoms suggestive of premature senility) have revealed key cancer suppression pathways, leading to the discovery of critical genome stability genes participating in processes of DNA repair, DNA replication, cell-cycle checkpoints and chromosome structural maintenance/segregation (see review in this issue by Martin and Oshima, pages 263–266). Many of these genes encode DNA polymerases and helicases as well as core components of the machinery for DNA mismatch repair, base excision repair, transcription coupled repair and non-homologous end-joining. The increased incidence of cancer associated with germ-line inactivation of the Werner helicase (WRN) and related helicases is particularly noteworthy as it underscores the inter-relationship of ageing, genome maintenance and cancer.

Curtis and Crowley[26] were the first to provide experimental evidence for an increasing level of genomic abnormalities as a function of age, reporting an increased number of abnormal metaphases in older, hepatectomized mice compared with younger animals (75% versus 10% aberrant metaphases, respectively). Similarly, aneuploidy, translocations and end-to-end fusions (dicentrics) have been shown to be higher in peripheral blood lymphocytes and fibroblasts of elderly humans compared with younger individuals[27–29]. An increase in chromosomal anomalies has also been observed in ageing mice, but the number of chromosomal dicentrics remained low throughout life[30], a finding that probably relates to species differences in telomere dynamics (see below). From these studies, it is clear that the process of normal ageing *per se* is associated with an overall deterioration in genome integrity, which might reflect an age-related decline in repair capabilities, or merely imperfect repair in the setting of age-dependent accumulation of mutations.

Several groups have provided some experimental data in support of the existence of declining repair capacity. Specifically, an age-related decline in the ability to process new ultraviolet (UV) light-induced DNA damage (measured by the ability to repair a UV-treated reporter plasmid) has been demonstrated in cultured primary skin fibroblasts and lymphoblastoid cell lines from normal donors in their first to tenth decade of life[31]. This age-associated decrease in the repair of UV-induced DNA damage coincides with decreased levels of proteins that participate in the repair process, such as ERCC3 (for excision repair cross-complementing 3), PCNA (for proliferating cell nuclear antigen), RPA (for replication protein A) and p53 (ref. 32), and with increased incidence of skin cancer in the aged. However, others have found no significant age-related differences in repair-replication response of human epidermal keratinocytes to UV-induced DNA damage[33]. The lack of an assay for the quantitative measurement of *in vivo* repair in humans has made this a difficult question to answer definitively.

Regardless, age-dependent changes in DNA repair proficiency have not been established unequivocally as a mechanism driving carcinogenesis in the aged. However, germ-line defects in mismatch repair can drive late-onset epithelial carcinogenesis in a subset of the general population. This has become apparent by the recognition that germ-line MutS homologue 6 (MSH6) mutations (a less critical component of the mismatch repair complex) are associated with colon cancers, although their late onset had led to the erroneous assignment of these hereditary cancers as 'sporadic'[34]. Although it is possible that genomic screens of sporadic adult cancers will uncover additional examples of 'low-penetrant' DNA repair alleles, most colon cancers as well as other carcinoma types do not exhibit the signature 'MIN' genomic profiles (that is, microsatellite instability resulting from mismatch repair deficiency) associated with DNA repair defects[19], implying that other mechanisms exist to drive epithelial carcinogenesis in the aged.

Epigenetic mechanisms and carcinogenesis

The lack of complete concordance between mutational rates and cancer profiles in the aged indicates that gene function and/or regulation might be affected by means of a non-structural mechanism. One class, termed epigenetic mechanisms, have been shown to operate primarily on the levels of DNA methylation and chromatin structure. Growing evidence exists that these epigenetic mechanisms modulate many different genes in an age-dependent and tissue-specific manner. *De novo* hypermethylation of CpG-rich islands (regions rich in cytosine–guanine doublets) nested in gene promoters has been shown to be a common means of silencing tumour-suppressor genes in cancers[35,36]. The observation that age-progressive CpG-island methylation takes place in a subset of cells residing in normal tissues and seems to operate in a gene-specific manner raises the possibility of targeting cancer-relevant pathways[37]. Issa and colleagues have proposed that this rising tide of promoter methylation in such genes might presage the intensification of methylation of these target sequences coincident with progression towards full malignant transformation[38]. That some of these methylated genes (for example, oestrogen receptor) have plausible links to growth control and oncogenesis has fuelled speculation that age-progressive methylation contributes to the silencing of cancer-relevant genes and hence to increased cancer incidence in the aged.

This hypothesis is supported by the genetic observation that the net growth rate and multiplicity of intestinal adenomas in the Min mouse (germ-line *APC* mutation) are reduced with loss of one copy of the prototypic DNA methyltransferase, Dnmt1 (refs 39, 40). On the other hand, the somatic disruption of DNMT1 in cultured human colorectal carcinoma cells, although associated with an overall decrease in genomic methylation, did not prevent methylation and silencing of many loci including the p16INK4a tumour-suppressor gene, thus pointing to the existence of other DNA methylating activities and their regional specificity of action[41]. However, the impact of DNMT1 disruption, or more generally of reduced genomic methylation, in pre-neoplastic cells might be very different from that in transformed cells. A detailed view of the molecular machinery responsible for these gene-specific methylation patterns and how the activity of this machinery is regulated in ageing tissues remain critical challenges for the future.

Telomere dysfunction and epithelial carcinogenesis

Age-dependent genetic and epigenetic events are likely contributors to the increased incidence of cancer in later life. Less evident is how such processes spur the preferential development of epithelial cancers in older humans, while sarcomas and lymphomas predominate in the paediatric population (and in mice at any age). Known tissue- and species-specific differences in the rates and types of somatic mutation seem insufficient to account for these observed cancer patterns. On a similar note, it is not clear whether differences in the relative prominence of key tumour-suppressor and repair pathways in humans compared with mice would engender these age and/or species variances (for review, see ref. 42). Moreover, these mechanisms do not readily explain one of the most distinctive

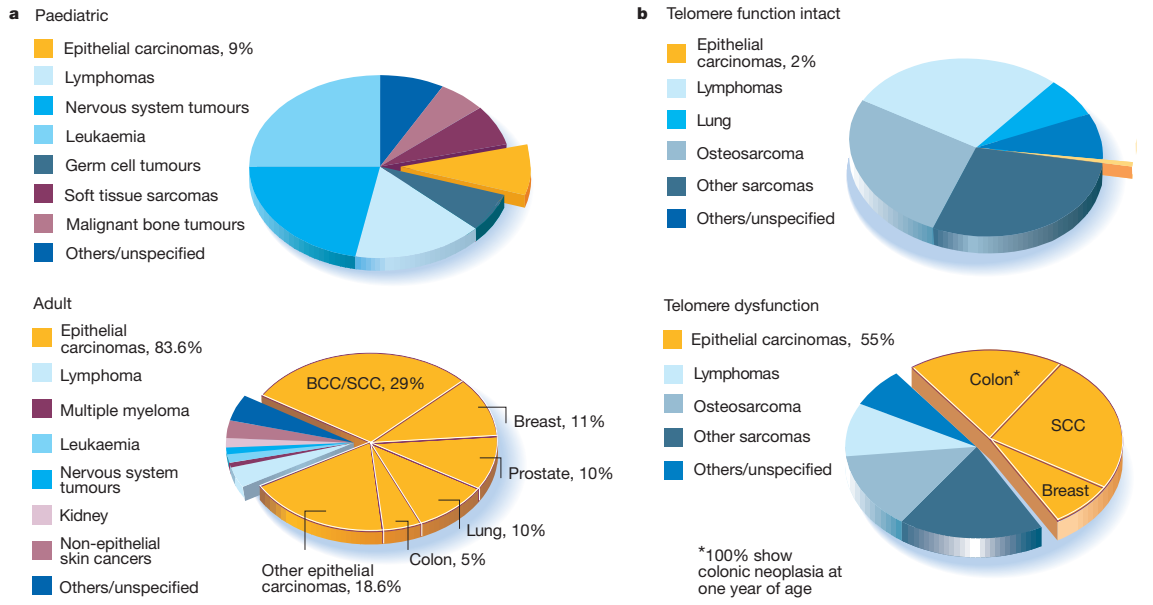

Figure 2 Tumour spectrum in human and mouse. **a**, Tumour spectra in humans represented in pie charts. Top chart includes the paediatric population; bottom chart the adult (male + female) population. Note the marked increase in proportion of epithelial carcinomas (orange-shaded segments) in the adult population. Compiled based on data in ref. 1. For basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), an estimated total of 500,000 cases is used[95]. **b**, Clinically apparent tumour spectra in mouse with and without functional telomeres, represented in pie charts[44]. Epithelial carcinomas are included in the orange-shaded segments.

features of epithelial carcinomas in ageing humans, as opposed to paediatric cancers and virtually all mouse cancers (including occasional epithelial carcinomas) — a radically abnormal cytogenetic profile typified by marked aneuploidy and complex non-reciprocal translocations[43].

A recent study using the telomerase-knockout mouse has indicated that differences in telomere length and regulation might impact dramatically on both the spectrum and cytogenetics of tumours during ageing[44]. Specifically, ageing telomerase-deficient mice, heterozygous for mutant p53, exhibited a pronounced shift in their tumour spectrum to carcinomas of the breast, colon and skin (Fig. 2). Importantly, these cancers emerged with cytogenetic profiles typical of human carcinomas. Are these data from the telomerase-deficient mouse relevant to mechanisms of epithelial carcinogenesis in aged humans? The answer has yet to be determined but recent cytogenetic and telomerase data from staged human epithelial cancers, particularly breast and colon cancers, makes the telomere–carcinoma connection an intriguing concept.

Telomeres comprise the nucleoprotein complexes that cap the ends of eukaryotic chromosomes and are maintained by the specialized reverse transcriptase, telomerase[45]. In human cells, insufficient levels of telomerase lead to gradual telomere attrition with passage in culture[46] and possibly with ageing and tumorigenesis *in vivo*[47–49]. Telomeres in human cancer cells are often significantly shorter than their normal tissue counterparts, indicating that telomere attrition occurs during the formative stages of cancer development where cell division takes place in the setting of low or absent telomerase activity[47]. The subsequent reactivation of telomerase would restore telomere function, albeit at a shorter set length. In the mouse, significant telomere shortening and dysfunction does not take place owing to somatic telomerase expression and long telomere lengths[50–54]. The observation of a more 'humanized' tumour spectrum and cytogenetics in mice engineered to experience age-dependent telomere attrition has led to the speculation that telomere dysfunction might be one of several mechanisms driving epithelial carcinogenesis as humans advance in age (Fig. 2).

Epithelial carcinomas derive from cell lineages that undergo continual renewal throughout life. Against this backdrop of presumed age-dependent telomere attrition, somatic mutations driving aberrant epithelial proliferation would lead to an overall shorter telomere length. If somatic mutations also neutralize the retinoblastoma/INK4a/p53-dependent senescent checkpoint, then continued growth beyond the Hayflick limit (the life span of normal fibroblasts *in vitro*) coupled with progressive telomere erosion would culminate in 'cellular crisis' – a period of severe telomere dysfunction accompanied by rampant genomic instability and massive cell death[55]. This dysfuctional telomere-induced genomic instability (or telonomic instability), combined with reactivation of telomerase, might enable a subset of post-crisis cells to emerge with a genetic profile permissive for malignant progression. In this manner, telomere-based crisis could provide the means to rapidly generate mutations necessary to initiate carcinogenesis. Subsequent reactivation of telomerase would serve to quell severe chromosomal instability and generate a more stable genome wherein the accumulation of mutational events further drives advanced stages of tumour progression. Such a transient period of explosive chromosomal instability could help generate the relatively high number of mutations thought to be required for adult epithelial carcinogenesis[14,15]. Moreover, telomere dysfunction and associated formation of dicentrics would set in motion fusion–bridge–breakage cycles[56], a mechanism capable of producing rapid and widespread changes in gene dosage as well as complex cytogenetics[44] (Fig. 3).

This telonomic instability model of epithelial carcinogenesis fits well with what is known about the timing of telomerase activation and evolving genomic changes during various stages of human carcinoma development, particularly those of the breast and

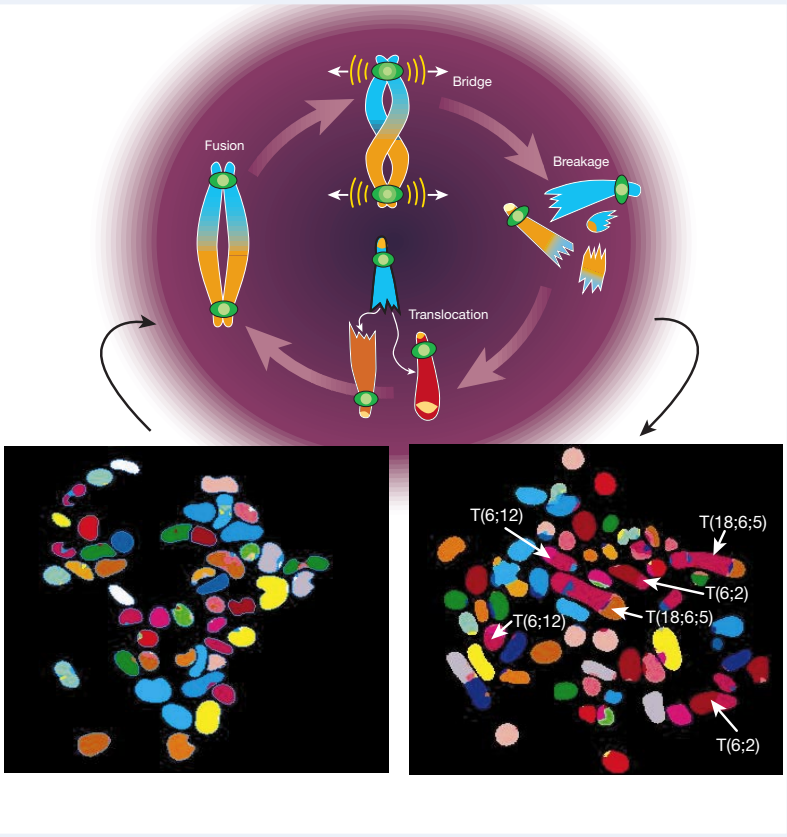

Figure 3 Fusion–bridge–breakage mechanism and cytogenetic profiles. Fusion–bridge–breakage process leads to chromosomal fragmentation and non-reciprocal translocations (top). Spectral karyotype (SKY) profile of mouse tumour cells with functional telomeres on left and with dysfunctional telomeres on right.

colon (Fig. 4). Comparative genome hybridization (CGH) has shown that human breast and colon tumours sustain widespread gains and losses of regions of chromosomes early in their development — such ploidy changes detected by CGH correlate well with the presence of complex chromosomal rearrangements[57–59]. This phase of genomic instability is evident by the carcinoma-*in-situ* stage of breast cancer and the early adenoma stage of colorectal cancer[20]. As these cancers progress through invasive and metastatic stages, genomic instability continues, apparently at a moderate rate[57–59], but is predicted to occur through non-telomere-based mechanisms. Correspondingly, the measurement of telomerase activity in adenomatous polyps and colorectal cancers has established that telomerase activity is low or undetectable in small and intermediate sized polyps and increases markedly in large adenomas and colorectal carcinomas[60]. Therefore, it seems that there is widespread and severe chromosomal instability early on during human tumorigenesis at a time when telomerase activity is low. However, it is worth emphasizing that a role for telomere dysfunction in human epithelial carcinogenesis will require an analysis of chromosomal/telomere status and telomerase activity levels in the same tumour samples from humans. Such studies are needed to fortify a causal link between telomere dysfunction and early chromosomal instability in human neoplasms.

Why should the pro-tumorigenic aspects of telomere dysfunction exert their action most prominently in self-renewing epithelial compartments and not contribute to an equivalent increase in other highly proliferative lineages such as lymphoid cells or mesenchymal lineages? One possible factor may relate to the extreme sensitivity of lymphoid cells to even modest levels of telomere dysfunction — impaired mitogenic responsiveness and increased apoptosis of lymphocytes is observed in young fourth-generation telomerase RNA component (mTERC)-null mice[61]. In contrast, compromise of gastrointestinal epithelium, as evidenced by intestinal villus atrophy, becomes apparent only in aged sixth-generation mTERC-null mice[62]. It is intriguing that telomerase is more readily upregulated following stimulation of normal lymphocytes[63] and that Myc, a potent regulator of telomerase, is invariably dysregulated in lymphoid malignancies[64]. Thus, the ready activation of telomerase and consistent Myc dysregulation could avoid telomere-based crisis during lymphomagenesis and might also explain their less complex cytogenetic profiles of paediatric lymphoid malignancies. With regard to mesenchymal tumours, the lack of a pronounced increase could presumably relate to the relatively low proliferative rate of these cell types, leading to reduced levels of age-dependent telomere attrition as well as a reduced expansion of stem/progenitor cells bearing a mutational load.

Finally, this model of telomere-related instability provides a rational explanation for the high incidence of cancer associated with diseases characterized by chronic cell destruction and renewal. One of the most notable examples of this strong link is the high incidence of hepatocellular carcinoma in late stage cirrhotic livers in which decades of continual hepatocyte destruction and regeneration are associated with a reduction of telomeres to a critically short length[65–68]. Such observations have raised an unanticipated therapeutic opportunity in that early somatic reconstitution of telomerase could attenuate telomere attrition and paradoxically reduce the occurrence of cancers in high-turnover disease states[65], a supposition that will require additional preclinical studies.

Tumour biology in the ageing organism

Malignant transformation represents the phenotypic end-point of successive genetic lesions impacting on functionally diverse cancer-relevant pathways. For virtually all cancer cells, there seem to be certain rites of passage on the path to transformation that include loss of cell-cycle control as a result of disruption of the retinoblastoma pathway[69], neutralization of a p53-dependent apoptotic

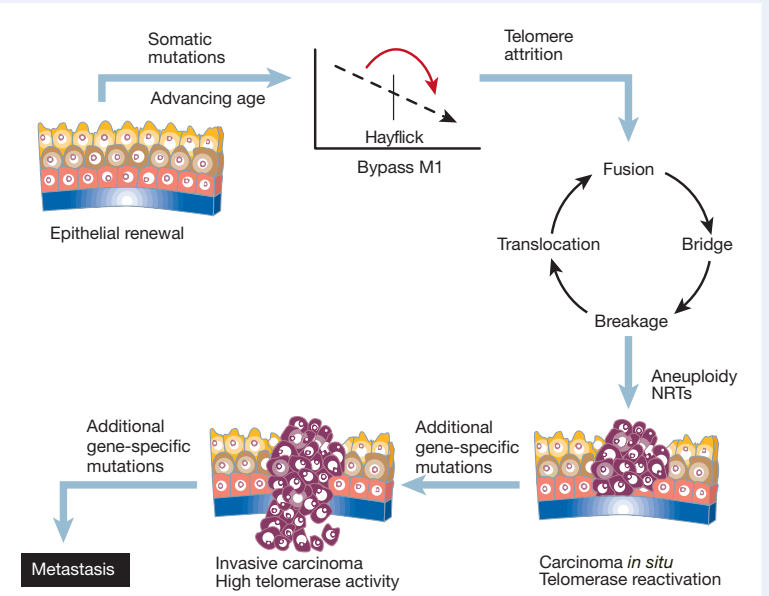

Figure 4 Dysfunctional telomere-induced genomic instability model of epithelial carcinogenesis. Continuous epithelial turnover during ageing is thought to lead to telomere shortening. When coupled with somatic mutations inactivating retinoblastoma/INK4a/p53 checkpoints, the Hayflick limit (mortality stage 1 (M1) or replicative senescence) can be bypassed. Continuous proliferation beyond the Hayflick limit results in progressive telomere attrition and subsequent fusion–bridge–breakage cycles in cells with dysfunctional telomeres. This process culminates in aneuploidy and complex non-reciprocal translocations (NTRs), resulting in massive and rapid changes in gene dosage observed in early carcinogenesis. Telomerase reactivation in the carcinoma-*in-situ* stage leads to relative chromosomal stability, providing a genome in which additional gene-specific mutations arise that are critical for progression to invasive and metastatic stages.

response elicited by this aberrant proliferation[70–72], long-term maintenance of chromosomal integrity[73–76], and establishment of a pro-tumorigenic microenvironment (reviewed in refs 16, 77). The latter transition is less well understood but has become increasingly recognized as a critical aspect of the tumorigenic process.

Complex homotypic and heterotypic interactions take place between tumour and host cells. The fully malignant tumour cell must become competent at instructing the host to establish a permissive and supportive environment for tumour growth and maintenance, including instructions to grow new blood vessels or to ignore tumour-associated antigens. The concept that genetic lesions intrinsic to the cancer cell drive and sustain these pro-tumorigenic host responses has received direct experimental support from inducible mouse models of cancer in which tumour-derived oncogenic signals were shown to be continually required for survival of host-derived endothelial cells[16,78,79]. Pro-tumorigenic signals emanating from the normal host compartment are less well characterized, nor is it clear whether this compartment evolves, with advancing age, towards one that is more permissive for carcinogenesis. One recurring example of a host-derived change might be a decline in immune function, one that would allow for increased escape of tumour cells from immune surveillance. This notion is consistent with a lower incidence of lymphomas in ageing mice exhibiting increased immune vigour[80], although these linkage studies do not rule out the possibility of having a tumour modifier linked tightly to the major histocompatibility complex locus. Is there evidence for the existence of other cancer-relevant age-dependent host factors, beyond possible immune deterioration? Some answers may lie in the complex interactions among stroma, epithelium and inflammatory cells.

Stromal–epithelial interactions are known to be crucial in the growth, survival and differentiation of normal and neoplastic epithelial cells. Fibroblasts are a major constituent of the stromal compartment and exhibit a number of age-dependent changes (see below). The extent to which these changes influence the stromal environment and affect the threshold for epithelial carcinogenesis is not yet known. However, an integration of several observations from the fields of ageing, development and tumour biology raises some intriguing possibilities. First comes the evidence that stromal cells exert a profound effect on tumorigenesis, particularly epithelial carcinoma[81]. In an elegant series of reconstitution experiments, Cunha and colleagues assessed the explant tumour growth derived from fully transformed prostatic epithelial cells admixed with fibroblasts isolated from either prostate cancer stroma or normal prostatic stroma[82]. Only prostatic carcinoma-associated fibroblasts were shown to support the tumorigenic growth of fully transformed prostatic epithelial cells *in vivo*[82], indicating that non-transformed stromal cells derived from the tumour microenvironment have either lost the capacity to exert suppressive control over fully initiated epithelial cells and/or acquired new capabilities permissive for tumorigenesis.

The signals responsible for 'reprogramming' of non-transformed stromal fibroblasts are not known and presumably emanate from tumour cells, although another, not mutually exclusive, possibility is that age-dependent processes *per se* entrain stromal cells. The latter notion follows from the observed accumulation of senescent dermal fibroblasts over time[83] and their increased production of cytokines, proteases and other matrix-degrading enzymes[27,84,85]. The factors driving fibroblast senescence *in vivo* have not been identified but could be analogous to those evoking senescence in cultured cells such as mitotic misregulation[27], aberrant oncogenic signals[86], and/or intrinsic DNA damage[87].

Regardless of the signals involved, the accumulation of senescent stromal cells with ageing, coupled with altered expression and elaboration of proteases and cytokines, has been hypothesized to create a tissue microenvironment that is more permissive for the growth of oncogenically transformed epithelial cells[85]. This hypothesis, proposed first by Campisi, seems more attractive in the light of a series of landmark studies of tumour biology in two transgenic mouse models of cancer[88–90]. In these studies, Hanahan and colleagues have shown that the matrix metalloprotease, MMP-9/Gel-B, acts as a key paracrine regulator of carcinogenesis. Furthermore, the effect of MMP-9 was reconstituted by bone-marrow transplantation onto an MMP-9 gene-knockout background, providing evidence for the sufficiency of MMP-9 and the fact that inflammatory cells serve as the main source of MMP-9. These model systems have also revealed that MMP-9 acts by mobilizing a latent store of vascular endothelial growth factor, the most potent endothelial cell mitogen and survival factor, thereby promoting the angiogenic switch critical for tumour progression[89]. It is worth noting that, although expression of MMP-9 has not been examined, several enzymes involved in extracellular matrix remodelling (for example, interstitial collagenase and stromelysin) are overexpressed in senescent fibroblasts[27,84,91].

Moreover, overexpression of MMP-3/stromelysin-1 by the stroma has been shown to promote mammary carcinogenesis[92]. These experimental observations, combined with 'stromal senescence', raise the question of whether age-dependent changes in the stromal milieu could contribute to tumorigenesis, specifically angiogenesis, in part by modulating inflammatory cell recruitment and behaviour (through elaboration of stroma-derived cytokines) or by acting in concert with inflammatory cells through increased local production of protease.

Summary, challenges and opportunities

Here I have reviewed some of the issues that are relevant to the connection between ageing and cancer and have speculated on how various age-sensitive molecular and physiological processes could act in concert to promote cancer, specifically epithelial carcinogenesis. Current data are consistent with, but not proof for, the combined pathogenetic effects of mutation load, epigenetic regulation, telonomic instability and altered stromal milieu. When considered collectively, these processes form a scenario in which continual epithelial renewal and somatic mutations (which disable the senescence checkpoint) allow for unrestrained growth and telomere attrition, culminating in a fusion–bridge–breakage–translocation process and marked genetic alterations. The relative resistance of epithelial cells to early telomere crisis, combined with the frequent loss of p53 in epithelial neoplasms, would serve to expand the pool of crisis cells from which a pro-cancer genotype would emerge. After reactivation of telomerase, this initiated cancer cell population incurs additional mutations essential for progression towards full transformation. The capacity of malignantly transformed epithelium to generate a mature tumour would also be governed by the tumour cell's ability to overcome the suppressive stromal milieu, a barrier that could be breached more readily in cytokine/protease-rich senescent stroma.

Each of these mechanisms, from molecular to cellular to organismal, require further verification through rigorous experimentation but their pursuit should uncover new paradigms from which rational therapeutics will emerge. In each of the areas presented in this perspective, there exist critical issues that need to be addressed. Mutation-rate analysis will benefit from improved *in vivo* methods that will determine accurately and comprehensively the frequency and spectrum of mutations in normal human cells and tissues, particularly in tissue stem cells. The development of equivalent assays in the mouse should provide comparative cross-species information that will contribute to our understanding of the basis for the distinct tumour spectrum seen in ageing humans and mice. In the area of epigenetics, unmet challenges include the needs for a detailed understanding of the methylation machinery and how its dysregulation contributes to carcinogenesis, a more comprehensive genome-wide view of the genes and loci subject to CpG-island methylation in cancer cells, and a determination of whether and how DNA methylation influences genome stability[93]. The role of telomere dysfunction in human epithelial carcinogenesis remains unproven. As such, it will be important to document telomere attrition in renewing epithelial stem cells and to perform a simultaneous comparison of telomere status, telomerase activity and chromosomal instability, particularly during the earliest stages of human epithelial cell transformation. Finally, the hypothesis that senescent stroma might facilitate epithelial carcinogenesis should be testable in reconstitution studies that assess the malignant potential of initiated epithelial cells admixed with either senescent or young stromal cells.

Cancer extracts a significant social and economic toll. The American Cancer Society estimates that, in the Unites States, 1,220,100 new cancer cases will be diagnosed in the year 2000 and nearly 80% of all cancers will arise in individuals aged 55 and older[1]. Each day more than 1,500 individuals succumb to this disease[1], most after a protracted and expensive clinical course. As the number of older individuals increases steadily in the population, the development of effective chemopreventive measures directed squarely against epithelial carcinogenesis assumes increasing significance. Productive clinical advances will no doubt flow from an improved understanding of the molecular and cellular mechanisms driving cancer in aged epithelium. A view of such mechanisms is coming into focus but many important details have yet to be revealed. It is with high confidence that the cancer research community anticipates that mechanistic insights will in turn lead to the design of effective therapeutics for prevention and for preferential tumour cell death — therapeutics that promise to convert this age of cancer to one concerned solely with longevity. □

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