European Journal of Cell Biology 102 (2023) 151340

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Clones of aging: When better fitness can be dangerous

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ARTICLE INFO

Keywords:
Aging
Clones
Clonal expansions
Growth pattern
Cancer

ABSTRACT

The biological and clinical significance of aberrant clonal expansions in aged tissues is being intensely discussed. Evidence is accruing that these clones often result from the normal dynamics of cell turnover in our tissues. The aged tissue microenvironment is prone to favour the emergence of specific clones with higher fitness partly because of an overall decline in cell intrinsic regenerative potential of surrounding counterparts. Thus, expanding clones in aged tissues need not to be mechanistically associated with the development of cancer, albeit this is a possibility. We suggest that growth pattern is a critical phenotypic attribute that impacts on the fate of such clonal proliferations. The acquisition of a better proliferative fitness, coupled with a defect in tissue pattern formation, could represent a dangerous mix setting the stage for their evolution towards neoplasia.

1. Foreword

A most intriguing advancement in our understanding of the biology of aging relates to the finding of pervasive clonal expansions in tissues with normal histological appearance. Initially described in hematopoietic cell lineages, this phenomenon has now been reported in several other tissues, including skin, oesophagus, liver, endometrium, brain, cardiovascular endothelium, bronchus, urothelium, prostate, large intestine and placenta (Cooper et al., 2015; Enge et al., 2017; Evans and Walsh, 2023; Florez et al., 2022; Lee-Six et al., 2019; Li et al., 2020; Martincorena et al., 2018, 2015; Moore et al., 2020; Sano et al., 2020; Yokoyama et al., 2019; Yoshida et al., 2020; Zhu et al., 2019).

The biological significance of these aberrant clonal expansions is being intensely discussed (Risques and Kennedy, 2018). The most immediate interpretation is that they help explaining the long-standing association between aging and neoplastic disease, given the presence of putative cancer-driver genetic alterations in at least some of these expanding clones. However, there is increasing awareness that such an appealing and straightforward view is too narrow and oversimplistic, in that the roots of oligoclonal outgrowths and their impact on the physiopathology of aging tissues go far beyond the origins of neoplastic disease.

2. Clones of normal aging

2.1. Clonal mosaicism in aged tissues

One of the first accounts that aging is associated with changes in tissue composition resulting from the slow prevalence of specific cell clones was provided by studies on X-inactivation patterns in peripheral white blood cells from normal human females of different age groups (Busque et al., 1996). It was reported that the incidence of skewing in X-chromosome (parental allele ratios $\geq$ 3:1) was 8.6 % in neonates and 16.4 % in individuals between 28 and 32 years of age, rising to 37.9 % in women aged $\geq$ 60 years. With more stringent criteria (considering allele ratios $\geq$ 10:1), the incidence of skewing was 1.9 %, 4.5 % and 22.7 % in the same 3 age groups, respectively. Over the past decade, the presence of age-related clonal haematopoiesis has been firmly established by several studies (Jaiswal and Ebert, 2019; Shlush, 2018). Clonal haematopoiesis of indeterminate potential specifically refers to the presence of clones harbouring a driver mutation at a variant allele frequency $>$ 2 % (Steensma et al., 2015). Its prevalence has been estimated to be over 20 % in individuals aged 60–70 years (Shlush, 2018).

It soon became clear that such an intriguing biological scenario is not unique to bone marrow-derived cell lineages but it is of common occurrence in many other tissues (Fig. 1). In a pioneering report, the eyelids of sun-exposed individuals were found to be disseminated with a high burden of mutant clones, including some harbouring multiple cancer-driver mutations, while maintaining the physiological functions of epidermis and a seemingly normal histology (Martincorena et al.,

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https://doi.org/10.1016/j.ejcb.2023.151340
Received 28 February 2023; Received in revised form 29 May 2023; Accepted 5 July 2023
Available online 6 July 2023

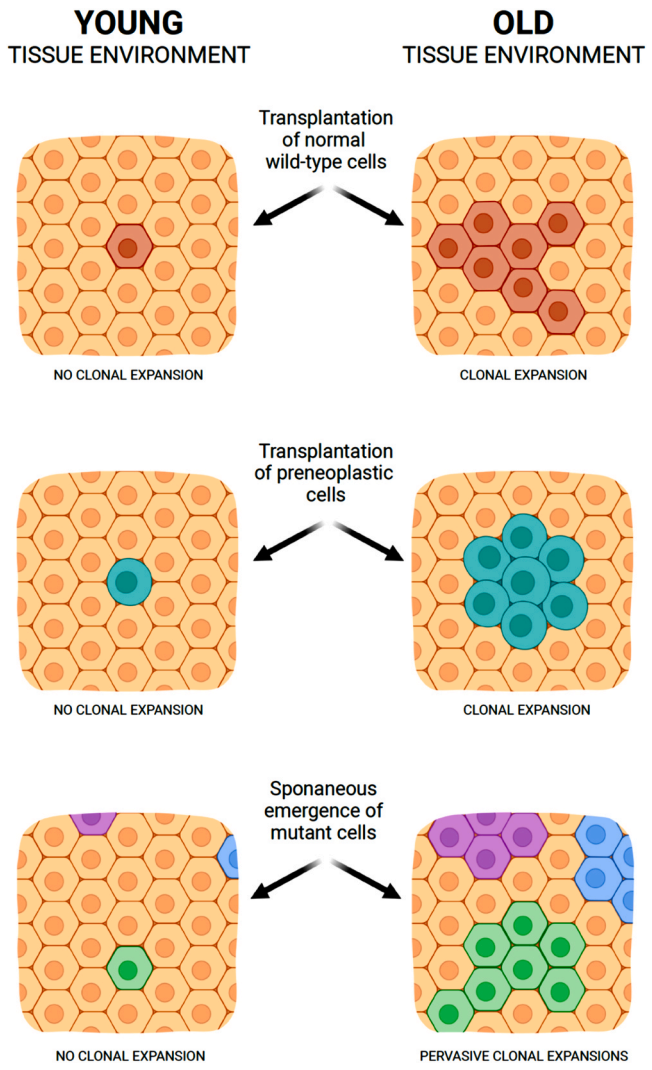

Fig. 1. The aged tissue environment (right panels) supports the clonal expansion of transplanted normal cells (top), transplanted pre-neoplastic cells (middle) and spontaneously emerging mutant cells (bottom).

2015). In the following years the presence of age-associated clonal expansions was described in the oesophagus, the liver, the brain, the endometrium, the bronchus, the urothelium, the large intestine, the vascular endothelium and the placenta. (Coorens et al., 2021; Evans and Walsh, 2023; Kakiuchi and Ogawa, 2021; Sano et al., 2020).

2.2. *The clonogenicity of the aged tissue environment*

Relevant to the above, transplantation studies revealed that the microenvironment of a normal aged tissue is supportive for the clonal expansion of both normal and preneoplastic cells. When normal hepatocytes isolated from a young donor rat were injected into the liver of either young or aged (18 month old) syngeneic hosts, they formed larger clusters in the latter compared to the former (Pasciu et al., 2006). Similar findings were reported following transplantation of foetal liver cells: they grew 4- to 5-fold more efficiently upon injection into old vs. young recipients (Menthena et al., 2011). Furthermore, pre-neoplastic hepatocytes isolated from liver nodules and transferred into rats of different ages displayed a phenotypic behaviour that paralleled that of normal counterparts: they formed large clusters and discrete nodules in older hosts, while limited growth was seen in young recipients (Marongiu et al., 2016) (Fig. 1). These data provide important insights towards elucidating the mechanistic bases of spontaneous clonal expansions associated with aging. It is noteworthy that caloric restriction (CR), which is known to delay aging and age-related diseases (Anderson and Weindruch, 2010), was able to decrease the clonogenic potential of the aged liver microenvironment on the growth of transplanted preneoplastic hepatocytes (Cadoni et al., 2017). To our knowledge, no studies have so far investigated the effect of CR on the emergence of age-associated mutant clones. However, it was reported that CR reduces the accumulation of spontaneous somatic cell mutation in aging rats (Aidoo et al., 2003).

3. Clones of aging and the need to count (quantity control)

The finding that aged tissues are frequently composed of a patchwork of evolving clones (Martincorena et al., 2015) is of utmost significance, representing a breakthrough in our understanding of the biology of aging and raising in turn several fundamental questions. A basic issue pertains to the nature of these clones with reference to homoeostatic mechanisms overlooking tissue mass and/or function. Albeit poorly understood, such mechanisms are normally and continuously operating to maintain defined reciprocal balances among cell/tissue types in our bodies. As an example, following haemorrhage bone marrow increases its activity and provides more differentiated cells to tip the transient deficit at peripheral sites. Conversely, hypertrophy and/or hyperplasia of any tissue give way to the removal of the excess mass/cells once the inciting stimulus subsides (Sarraf et al., 1997). Such need to count is a defining feature of complex multicellular organisms, ensuring that the differentiated contributions of each cell/tissue type to the integrated bodily functions are quantitatively appropriate. Are age-associated clonal expansions included in this counting or are they growing above and beyond the limits imposed by homoeostatic control mechanisms? How one answers this question is crucial, in that it will inform the biological interpretation of this phenomenon and it will orient research directions into its aetiology, pathogenesis and possible therapeutic approaches, if any (Marongiu et al., 2018). No studies allow to draw definitive conclusions on this issue, so far. A prevalent view is that the large majority of clones in aged tissues expand within physiological limits and are therefore to be considered as part of the normal cell turnover in that tissue (Fabre et al., 2022). Consistent with this, tissue histology is typically well preserved. On the other hand, the common presence of cancer-associated mutations in such clones could be taken as evidence to suggest that their growth may exceed tissue homoeostasis and may in fact be an early manifestation of growth autonomy, on the path to overt neoplastic disease.

4. Positive selection and clonal fitness (quality control)

4.1. *Random drift vs. positive selection*

Relevant to the issue discussed above, additional studies have provided important insights on the biological and molecular mechanisms leading to the emergence of clonal expansions in aged tissues. Positive selection, not drift, was found to be the major force shaping clonal haematopoiesis in the aged bone marrow, implying that a relatively higher fitness of the involved clones is sustaining the process (Watson et al., 2020). Similar findings have been reported in solid tissues: for example, strong positive selection of NOTCH1 and p53 mutant clones was documented in the human oesophageal mucosa, involving up to 80% of the lining epithelium (Martincorena et al., 2018).

4.2. *Context-dependent clonal fitness*

Central to positive selection is the concept of cellular fitness, which includes both cell-intrinsic and cell-extrinsic components (Watson et al., 2020). A purely cell-intrinsic increase in fitness is difficult to conceive, in that cell fitness is inevitably context-dependent (Watson et al., 2020). Hypothetically, under a similar scenario aged tissues would be slowly

repopulated by cellular clones displaying a higher-than-normal performing phenotype, at least in terms of regenerative potential. However, assessing (or even defining) fitness is not an easy task for any given tissue and cells endowed with higher-than-normal proliferative proficiency are not necessarily fitter in overall functional performance (Marongiu et al., 2021). Moreover, aging involves a generalised decrease, and not an increase, in the regenerative proficiency in several tissues (Brazhnik et al., 2020; Harrison and Astle, 1982; Maeso-Díaz et al., 2022), implying that even cells preserving a normal growth potential may be positively selected in that context.

Most often, a positive or negative impact on fitness can result from cell-extrinsic constraints that are imposed by the tissue microenvironment, favouring or disfavouring the emergence of specific cellular phenotypes (Laconi et al., 2020; Marongiu and DeGregori, 2022). Selected cells are not intrinsically fitter than surrounding counterparts by any absolute parameter, but they are better equipped to thrive under defined and specific conditions (Florez et al., 2022). A notable example to illustrate this situation is the selective expansion of *Dnmt3a*-mutant haematopoietic stem cells driven by mycobacterial infection in chimeric bone marrow model obtained via transplantation of Dnmt3a$^{-/-}$ and wild type (WT) haematopoietic stem cells (Hormaechea-Agulla et al., 2021). Remarkably, injection of recombinant IFNγ alone was sufficient to reproduce the selective effect of infection on *Dnmt3a*$^{-/-}$ clones. It was also established that reduced differentiation of mutant vs. WT clones was one of the biological mechanisms contributing to the selective emergence of the former (Hormaechea-Agulla et al., 2021). More recent studies have further supported the mechanistic association between inflammatory environment, ageing and clonal haematopoiesis. When WT or *Dnmt3a*-mutant haematopoietic stem cells were transplanted in sub-lethally irradiated mice of different age, mutant clones exhibited a growth advantage in aged recipients (Niño and Pietras, 2022). However, the selective advantage was abrogated in mice defective in TNF-α receptor signalling, suggesting that the increased levels of this pro-inflammatory cytokine in aged animals and individuals is fuelling, at least in part, the emergence of *Dnmt3a*-mutant cells.

Moreover, the chronic pro-inflammatory microenvironment of MDS, including elevated levels of cytokines and chemokines, leads to decreased self-renewal of normal bone marrow stem cells, while mutant clones with activated TLR-TRAF6 signalling and non-canonical NFκB pathway can better withstand this effect and selectively emerge (Muto et al., 2020). Notably, cell-intrinsic TLR-TRAF6 activation impaired hematopoietic stem cell function in mouse models (Fang et al., 2017), suggesting that the mutation per se does not confer any growth advantage over the WT phenotype under normal conditions. Such context-dependent selection of mutant phenotypes has been reported in several other systems. Thus, *p53*-mutant cells of the mouse oesophageal mucosa show a better fitness than their normal counterparts during exposure to oxidative stress induced by low dose ionising radiation (LDIR); however, such growth advantage is lost when LDIR is coupled with antioxidant treatment (Fernandez-Antoran et al., 2019). Similarly, high fat diet-induced inflammation selects for *RasV12*-mutant cells in the intestinal epithelium, while simultaneous treatment with aspirin attenuates this effect (Sasaki et al., 2018). Furthermore, it was reported that a population of bronchial epithelial cells that remain mitotically quiescent during exposure to smoking, thereby incurring a low mutagenic burden, resume proliferation and replenish large segments of mucosa upon smoking cessation (Yoshida et al., 2020). This suggested that cells with a near normal genotype display a better fitness compared to more damaged counterparts when exposure to smoking subsides.

5. Clones of aging and tissue turnover

Overall, the picture that takes shape putting together the tiles of this mosaic is consistent with the notion that clonal dominance associated with age is a direct consequence of normal dynamics steering cell turnover in our tissues, whereby the expansion of specific clones is largely dictated by (micro)-environmental cues selecting for the fitter phenotype (Zhu et al., 2019). Recent findings on the clonal dynamics of haematopoiesis across the human lifespan are supportive of this interpretation. While in individuals younger than 65 years the generation of blood cells is evenly contributed by 20,000–200,000 stem/progenitor cells, in those older than 75 years clonal diversity was profoundly decreased, with 30–60 % of blood cell output accounted for by very few (12–18) independent clones (Mitchell et al., 2022). Possible driver mutations were only identified in 22 % of these clones, implying that in over 3 out of 4 clones the genotype associated with better fitness was unknown (Mitchell et al., 2022). The conclusion is that a drastic decrease in clonal diversity should not be considered as a mere accidental, however frequent, phenomenon (Jaiswal et al., 2014), but should be taken as a normal, universal feature of haematopoiesis in aged humans; furthermore, it is sustained by the continuous positive selection of cellular clones whose genotype is yet to be identified (Jaiswal et al., 2014) and needs not be necessarily related to neoplastic disease.

6. Clones of aging and tissue function

6.1. Clones of haematopoietic tissue

A fundamental question emerging from the above findings pertains to biological and clinical consequences of age-associated clonal expansions, if any. The most intuitive association one is led to make in this regard is with neoplastic disease. In fact, clonal haematopoiesis was soon identified as a possible pre-neoplastic stage on the path to malignant transformation, fitting with the concept of carcinogenesis as a multistep process (Jaiswal et al., 2014) (more on this topic in the following paragraph). However, it was quite unsettling to discover that the presence mutant clones in peripheral blood cells also carried an increased risk of developing other chronic, age-associated diseases, whose list has been continuously expanding (see Florez et al., 2022 for a recent update). For example, the risk of atherosclerotic cardiovascular disease was found to double in people with clonal haematopoiesis (Jaiswal et al., 2014), although subsequent studies have reported higher (Dorsheimer et al., 2019) or lower (Kar et al., 2022) risk levels. Similarly, clonal haematopoiesis increased the frequency of adverse outcomes in chronic kidney disease (Dawoud et al., 2022) and imposed a higher risk of chronic obstructive pulmonary disease (Buscarlet et al., 2017). Importantly, the presence of mutant clones in peripheral-blood cells also increased all-cause mortality risk by a factor of 1.4 (Jaiswal et al., 2014). Furthermore, mouse models of hematopoietic-specific mutations in *Dnmt3a*, the most commonly mutated gene in human clonal haematopoiesis, display increased osteoporosis and increased osteoclastic activity; this was attributed to pro-inflammatory cytokines secreted by *Dnmt3a*-mutant macrophages (Kim et al., 2021). In addition, mice with a mutant *Tet2*, another frequent alteration of human clonal haematopoiesis, have increased expression of inflammatory genes in innate immune cells, accelerated atherosclerosis and enhanced development of pulmonary emphysema following lung inflammation (Dawoud et al., 2022; Dorsheimer et al., 2019; Jaiswal and Libby, 2020; Miller et al., 2022). This suggests that the presence of a pro-inflammatory environment fuelled by mutant clones could mediate, at least in part, the effects on mortality referred to above, including all-cause mortality (Jaiswal and Libby, 2020). On the other hand, it was reported that an inflammatory environment favours the emergence of *Tet2*-mutant haematopoietic clones (Abegunde et al., 2018), envisaging a possible feeding-forward loop in which mutant clones contribute to inflammation and the latter in turn selects for those mutants.

6.2. Clones of solid tissues

Compared with clonal haematopoiesis, relatively little information is available so far on functional consequences of the presence of mutant clonal expansions in solid tissues, apart from an increased risk of

neoplastic disease (discussed in the next section). At least two possibilities can be envisioned. Mutant clones, depending on their size, may impact cell/tissue function via qualitative/quantitative changes affecting the synthesis of biomolecules directly or indirectly related to the underlying genetic alteration. A prototype of this scenario is paroxysmal nocturnal haemoglobinuria associated with emergence of hematopoietic clones lacking phosphatidylinositol glycan class A gene, which is required to produce glycolphosphatidyl inositol anchors (Babushok, 2021).

Alternately, the presence of few dominant clones, which leads to a reduction in the number and diversity of cell lineages contributing to tissue composition, could in turn reduce tissue plasticity and adaptive capacity to environmental challenges, alterations that are typical attributes of advancing age. Along these lines, very recent intriguing findings have indicated that sleep, which protects against age-associated cardiovascular, neoplastic and neurodegenerative diseases (Besedovsky et al., 2019; Irwin, 2019) is able to programme the epigenome of hematopoietic progenitor cells and preserves clonal diversity and adaptability (McAlpine et al., 2022).

An intriguing case in point is endometriosis, consisting in the presence of endometrial epithelium and stroma at ectopic sites. While it is considered clinically benign, with virtually no risk of neoplastic transformation, it was nevertheless found to harbour frequent mutant clones, particularly in deep infiltrating lesions (Anglesio et al., 2017). Mutant cells, including clones with cancer driver mutations, are also common in histologically normal endometrium (Suda et al., 2018); however, they appear to undergo positive selection in the inflammatory microenvironment of endometriotic tissue (Anglesio et al., 2017; Suda et al., 2018).

7. Clones of aging and neoplastic disease

7.1. Mutant clones as cancer precursors

As mentioned above, it is almost axiomatic that the finding of mutant clonal expansions in healthy aged tissues is primarily suggestive of a link with neoplastic disease. When it was first reported in hematopoietic tissue, it was taken as a fundamental step towards a better understanding of the complex connection between aging and cancer (Jaiswal et al., 2014). Individuals harbouring age-associated, bone marrow-derived mutant clones were found to run an increased risk for the development of hematopoietic malignancies. Additionally, genetic alterations that are of common occurrence in such mutant clones are also frequently found in leukaemic cell populations, supporting a precursor-to-product relationship between the former and the latter (Jaiswal et al., 2014). Mechanistically, recent studies point to inflammation, fuelled by aberrant, pro-inflammatory monocytic cell clones, as a possible driver for the subsequent selection and emergence of blood cell neoplasms (Hormaechea-Agulla et al., 2021; Niño and Pietras, 2022; Yeaton et al., 2022). A similar paradigm might also apply to solid organs. Clones of p53 mutant epithelial cells are frequent in aging human oesophageal mucosa, and biallelic disruption of p53 encoding gene is commonly found in cancer of the oesophagus (Murai et al., 2022).

7.2. Mutant clones and cancer suppression

However, it is becoming increasingly clear that the attribution of a putative pre-neoplastic nature to the age-associated clonal expansions is far from capturing the full spectrum of their biological significance. Once again, studies conducted on bone marrow-derived cell population have provided crucial insights in this direction. As mentioned previously, analysis of clonal dynamics of haematopoiesis across the human lifespan has revealed that a drastic reduction of clonal diversity is a normal and constant finding in aged humans, resulting from the positive selection of several genetic variants, a large proportion of which is yet to be identified (Fabre et al., 2022; Mitchell et al., 2022). This reiterates the concept that clonal haematopoiesis associated with aging represents the result of normal cell turnover dynamics in this tissue, whereby a limited number of clones emerge through mechanisms of intrinsic and/or extrinsic cell competition. Within this framework, it is still uncertain whether and to what extent these emerging clones have any bearing on neoplastic disease.

The complexity of the issue is well illustrated by findings reporting the elimination of emerging tumours by outcompeting, mutant neighbouring clones in the oesophagus of mice (Colom et al., 2021). It was suggested that survival of early neoplasms was critically dependent on the competitive fitness of mutant and histologically normal surrounding epithelium, in that phenotypically normal cell clones, albeit harbouring genetic alterations, could act as a barrier against early tumorigenesis (Colom et al., 2021). Such scenario is in line with the concept of "decoy fitness peaks" proposed a few years ago, whereby the presence of fitter clones with a (near-)normal phenotype can halt or delay the selective emergence of other clones with (pre-)neoplastic potential (Higa and DeGregori, 2019). Similarly, mutations in *PKD1, PKDH1,* and *PPARGC1B* genes are common in clonal nodules of cirrhotic livers, while they are rarely found in hepatocellular carcinoma (Müller et al., 2019). It was proposed that the mutational landscape associated with chronic liver disease favours pathways that are related to proliferative/regenerative fitness and may limit the risk for the emergence of (potentially) malignant clones (Müller et al., 2019).

7.3. Clones of aging and neoplastic disease: phenotype matters

It is apparent that selective expansion of mutant clones per se is not necessarily geared towards cancer and may in fact exert a countering effect on the growth of early (pre-neoplastic) lesions). Thus, a relevant question to ask pertains to which critical phenotypic property/ies of expanding clones is/are associated with an increased risk of progression to overt cancer, i.e. the acquisition of growth autonomy, invasive and metastatic capacity. In a follow up study on the longitudinal dynamics and natural history of clonal haematopoiesis it was reported that mutations driving faster clonal growth carry a higher risk of malignant progression (Fabre et al., 2022). While this is an important insight for haematological malignancies, it is difficult to predict how applicable it might be to solid tissue neoplasms, given the more stringent constraints these cell populations need to overcome towards the acquisition of invasiveness and metastatic potential (Oppenheimer, 2006). In both the oesophagus and the epidermis, a common theme in the selective emergence of mutant clones lies in a bias in cell fate, so that mutant cell divisions produce an excess of progenitors over differentiated cells (Murai et al., 2018), a principle that appears to apply to bone marrow clonal dynamics as well (Hormaechea-Agulla et al., 2021). However, such bias favouring mutant cells decreases and reverts towards balance as expanding clones are surrounded by similar counterparts, allowing the tissue to retain its integrity (Murai et al., 2018).

8. Clones of aging and growth pattern

8.1. Clonal growth vs. focal growth

In the context of the above discussion, an often-neglected aspect of preneoplastic lesions in solid tissues pertains to their altered growth pattern, as indicated by the *focal* nature of such clonal expansions (Marongiu et al., 2012). Studies conducted by our research group on transplantation of isolated hepatocytes in a pre-conditioned tissue environment, (whereby the cell cycle of resident parenchymal cells was persistently blocked following chemical exposure), revealed a striking difference of normal vs. preneoplastic cells in their pattern of interaction with the host liver tissue. Clusters of expanding normal hepatocytes were macroscopically and histologically indistinguishable from the recipient parenchyma and were spatially oriented according to apical-basal domains of resident cells, with hybrid bile canaliculi

forming between transplanted and host hepatocytes (Laconi et al., 1998). By contrast, clones of preneoplastic hepatocytes did not integrate in the recipient liver, formed discrete focal/nodular lesions distinct from surrounding tissue and displayed altered cell polarity (Laconi et al., 2001).

8.2. Growth pattern in the pathogenesis of neoplastic disease

The hypothesis that alterations in tissue patterning can contribute to the pathogenesis of neoplastic disease is supported by several lines of evidence (Martin-Belmonte and Perez-Moreno, 2012; McCaffrey and Macara, 2011). Early observations in *Drosophila* indicated that mutants in *scribble (scrib)* gene, whose product is involved in cell polarity determination in the eye disc, cooperate with oncogenic *Ras* or *Notch* towards neoplastic development (Brumby, 2003). An additional finding was that proteins involved in the establishment of cell polarity were common targets of viruses associated with human carcinogenesis (Javier, 2008). Later, liver-specific KO mice for the Rho small GTPase Cdc42, which plays a critical role in cell polarity, were found to be prone to hepatic neoplasia (van Hengel et al., 2008). Furthermore, loss of the Par3 polarity protein was reported to promote breast tumorigenesis in mice (McCaffrey et al., 2012). Along the same vein, it was proposed that H. pylori-induced disruption of cell polarity, through chronic dysfunction of the small GTPases Cdc42/IQGAP1-signalling pathway, may contribute to the origin of gastric carcinoma in humans (Osman et al., 2013). Dysregulation of Cdc42 was also suggested as relevant to the development of human colorectal cancer (Leve and Morgado-Díaz, 2012) and basal cell carcinoma (Tucci et al., 2013). Later, lack of Cdc42 expression in mouse bronchial epithelium has been associated with loss of contact inhibition, disruption of cell polarity and increased tumour formation (Zheng et al., 2017). Interestingly, molecules associated with the Cdc42 signalling pathways were differentially phosphorylated upon chronic exposure in vitro to cigarette smoke (Solanki et al., 2017). Most recently, apical-basal polarity has also been implicated in determining stem cell number in the intestine of *Drosophila* (Wu et al., 2023).

The evidence above suggests that altered cell polarity, and the ensuing distortion in tissue pattern formation, are not mere bystanders of overt neoplastic disease, but they are often early events of possible pathogenetic significance (Stephens et al., 2018). We propose that an altered growth pattern represents one of the critical phenotypic features of age-associated clonal expansion which can confer upon them an increased risk for the evolution towards neoplasia (Fig. 2).

8.3. Linking growth pattern and neoplastic process

How could an altered tissue architecture contribute towards the emergence of an invasive and metastatic phenotype? Years ago, we called attention on a fundamental distinction that should be made between the tissue environment where early preneoplastic lesions develop and the tumour microenvironment inside focal lesions, such as polyps, nodules, adenomas (Laconi et al., 2008). While the former is the outcome of orderly developmental processes resulting in a functional cellular community with solid infrastructure (including stroma and blood vessels), the latter is often at risk of shortage in the supply of oxygen and metabolic substrates, as a consequence of defective wiring of the expanding lesion (Folkman, 1972). Numerous studies have in fact documented the presence of hypoxic conditions and/or decreased blood supply during carcinogenesis, starting from early stages (Bos et al., 2001; de Koster et al., 2022; Jiang, 2003; Kadonosono et al., 2011; SoIt and Hay, 1977; Thomlinson and Gray, 1955). This type of evidence lead Judah Folkman and his associates to propose that the "angiogenic switch", i.e. the ability to generate new blood vessels, represented a mandatory biological step in neoplastic progression that could be amenable to therapeutic targeting (Folkman, 2003; Gimbrone et al., 1972; Sherwood et al., 1971). However, hypoxia is also well known as a potent driver of tumour progression (Fang et al., 2008; Regan Anderson et al., 2013; Semenza, 2000; Shao et al., 2022), including the ability to induce cell migration, invasive and metastatic phenotypes (Graham et al., 1999; Sullivan and Graham, 2007). Thus, altered tissue architecture, which is basic to the morphological diagnosis of (pre-)neoplastic lesions and is axiomatically associated with the entire carcinogenic sequence in solid tissues, is also likely to play an active role in the pathogenesis of neoplasia through the induction of a specific microenvironment fuelling the process.

Based on these considerations, the fate of age-associated clonal expansions could critically depend on their growth pattern more than on their growth rate per se. As pointed out above, clones resulting from a bias in cell fate, with mutants producing an excess of progenitors over differentiated cells, revert to a balanced growth once they are surrounded by counterparts with a similar phenotype (Murai et al., 2018). Importantly these clones maintain normal morphology, normal turnover dynamics and normal overall tissue homoeostasis (Marongiu et al., 2021; Murai et al., 2018).

It is noteworthy that normal tissues have the ability to sense and eliminate cells displaying an aberrant growth pattern and/or altered cell polarity, in a process requiring the active presence of functional wild-type neighbours (Brown et al., 2017; Yamamoto et al., 2017). This indicates that such cells are perceived as disruptive for tissue structure and function, lending support to the hypothesis that they also represent a risk factor for the emergence of neoplastic disease. On the other hand, aging may reduce the efficiency of such surveillance mechanism, thereby unleashing the expansion of morphologically altered clones.

9. Conclusions

In summary, evidence is accruing that age-associated clonal expansions often result from the normal dynamics of cell turnover in our tissues. The aged tissue microenvironment is more prone to favour the emergence of specific clones with higher fitness partly because of an overall decline in cell intrinsic proliferative/regenerative potential of the surrounding counterparts (Bogeska et al., 2022; Jasper, 2020; Maeso-Díaz et al., 2022; Matsumura et al., 2016; Pentinmikko and

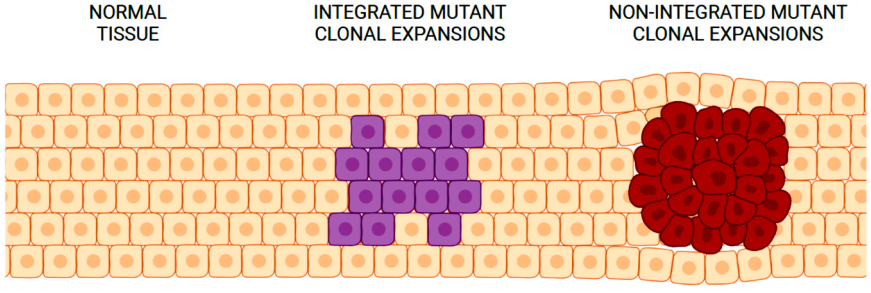

Fig. 2. Integrated vs. non-integrated clonal expansions: clones displaying a non-integrated growth pattern are more susceptible to neoplastic progression (see text for discussion).

Katajisto, 2020; Serra et al., 2015; Tao et al., 2020; Timchenko, 2009; Wang and Dreesen, 2018). For example, donor age is inversely related to the rate of regeneration following liver transplantation in humans (Ono et al., 2011). More to the point, hepatocytes with a normal phenotype form larger clusters upon infusion in the liver of aged compared to young syngeneic recipients (Menthena et al., 2011; Pasciu et al., 2006). This implies that expanding clones in aged tissues need not to be associated a priori with the development of cancer, albeit this is a possibility. On the other hand, the association of clonal haematopoiesis with other chronic ailments typical of old age, each with specific pathogenetic pathways, questions the almost axiomatic link that one is tempted to establish between clonal expansions per se and neoplastic disease (Marongiu and DeGregori, 2022). We suggest that growth pattern is a critical phenotypic attribute that impacts on the fate of such clonal proliferations. The acquisition of a better proliferative fitness, coupled with a defect in tissue pattern formation, could represent a dangerous mix setting the stage for their evolution towards neoplasia.

CRediT authorship contribution statement

All authors conceived and discussed the content of the manuscript. EL wrote the initial draft, FM and SC draw the figures and all authors were involved in the final editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

Acknowledgements

This work was funded in part by Fondazione di Sardegna (FM and EL, CUP F74I19001020007).

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