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Review

Evolution of Cancer Progression in the Context of Darwinism

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Abstract. *Our review compares evolution of cancer in the human body to the origin of new species from a common ancestor organism with respect to the theory of Charles Darwin. Moreover, the functional role of the tumor microenvironment as a selective pressure actively participating in cancer progression is also demonstrated. Evolutionary aspects of tumor growth and invasion from the point of view of modern therapeutic challenges and opportunities in precision personalized medicine are also discussed.*

Increased incidence of oncological disorders poses a major issue to public health worldwide. In particular, the incidence rates have increased in most countries since the second half of the 20th century (1). In recent years, cancer has become the second leading cause of death globally after cardiovascular diseases, responsible for approximately 9 million deaths per year. Although significant effort has been spent in the development of new drugs and personalized therapeutic approaches, which has raised high expectations and slightly improved patient survival, we must admit that the war against cancer has not yet been won (2, 3). It may be hypothesized that all cancers undergo a complex process of natural selection – similarly as described 158 years ago by Charles Robert Darwin in his extraordinary publication "The Origin of Species by Means of Natural selection" (4). Hence, evolutionary aspects of cancer progression in individual patients can help us better understand cancer biology and perhaps bring new therapeutic strategies resulting in improved health care and survival rates.

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Cancer: The Health Epidemic of the 21st Century

The increased incidence of cancer may be associated with several factors including environmental pollutants and changes in lifestyle. However, aging of the population is also substantially correlated with the remarkable increase of cancer burden observed in the population (5). Organisms have developed sophisticated molecular mechanisms to repair their damaged genes with reasonably high efficiency. This represents an investment in the stability of the genome and enables them to pass unaltered genetic information to the next generation. Any failure at this stage can lead to vanishing of affected genes from the population pool. When the repair program cannot be successfully executed, alternative machinery leading to cell death is preferred and rapidly initiated. This important mechanism can also prevent the later formation of clones from genetically altered cells – the foundation of future tumors. Unfortunately, the efficacy of this repair system significantly decreases with age. Of note, a decrease of fertility is also noted at this stage in life (roughly 55 years of age in humans). During the passing

years, all active and proliferating cells randomly accumulate mutations. This inevitable process results in the genetic instability frequently observed in the elderly. Even in non-dividing cells, for example in brain neurons, thousands of mutations per individual genome can be detected in octogenarians (6, 7). Therefore, it is not surprising that people at a higher age (above 60 years) suffer from malignancies of different nature and different biological behavior than do younger individuals. Based on available data, it is possible to interpret the remarkably high incidence of cancer in elderly people as being a consequence of a developmental and reproductive strategy of our species, *Homo sapiens*, where the gene repair machinery is systematically reduced in the elderly (8). If so, it is likely that many symptoms of aging such as cardiovascular disorders, muscle sarcopenia, cognitive problems and osteoarthritis are also associated with such a genetic imbalance (9, 10).

A Short Introduction to Darwin's Theory

The British biologist, geologist and naturalist Charles Robert Darwin (1809-1882) left the port of Plymouth (United Kingdom) on board H.M.S. Beagle in 1831 and returned from his trip around the world 5 years later. This journey ignited one of the most prolific movements of modern evolutionary biology. During the journey, the ship with Charles Darwin on board also landed at the Galapagos, the remote volcanic archipelago located in the Pacific Ocean, approximately 1,000 km from the nearest coast of Ecuador. Although Darwin spent only 5 weeks in the Galapagos, it fundamentally shifted his view on life on Earth and its diversity forever. Twenty-three years after the return home of H.M.S. Beagle, Charles Darwin published his evolution theory (https://www.britannica.com/biography/Charles-Darwin) (11).

Briefly, Darwin described his hypothesis of the common ancestor, whose offspring can vary during long periods of time. These phenotypic variants are clearly visible evidence of the developmental shift within a generation. If these traits prove to be beneficial for survival in the context given by the environment, they prevail, and a new species can be established consequently. The bottleneck effect is an extreme example of when the size of a population is severely reduced due to a sudden change of external factors leaving behind a small assortment of survivors. Darwin highly emphasized the role of sexual reproduction as a tool for the spreading of new phenotypes (traits) in abandoned environmental niche leading to an increase in the diversity of life on Earth. When reproductive isolation coincides with such newly occurring diversity, species can be readily established. This can be easily exemplified by the so-called Darwin's finches from the Galapagos. They remarkably differ in the shape of their beaks. The shape of the beak arises from its function, which is dependent on the predominant food type and its availability in various islands of the archipelago. The isolation of birds in remote islands well illustrated the process of formation of new species. In terms of modern molecular biology, all organisms have developed systems to repair gene errors, but its efficiency is not faultless. When mutation also affects the germ cells, it is carried through to offspring (12). When it is beneficial to the organism colonizing the distinct niche of the ecosystem, the mutation is fixed, and new species are developed from the ancestor (13, 14). Darwin's explanation well elucidated the role of the impact of the genotype, its effects on the position of the organism in the ecosystem and also on the development of new species (Figure 1).

The Genetic Heterogeneity of Tumors and Natural Selection

Although lethal infections are considered to be the most powerful natural selective forces, a similar selection mechanism also operates in multiple types of cancer, such as breast cancer, malignant melanoma, and acute lymphoblastic leukemia (15-17). Therefore, it is not surprising that tumors originally sensitive to a certain therapy frequently acquire resistance. On the other hand, natural selection has also resulted in several amazing outcomes including ourselves – human beings – and in not entirely developed rudimentary or atrophied organs, such as the *mammae* of male mammals. Despite the suggested clonal origin of tumors, a clinically manifested disease reveals remarkable cellular genetic heterogeneity that is the principal expression of growth, variation, differentiation and natural selection. Most importantly, the initial transformation of a cell may occur at the cancer progenitor or stem cell level and many of types of cancer develop by transformation of tissue-specific adult stem cells. Tumors, therefore, include therapy-sensitive cells along with cells which demonstrate partial or complete resistance (18, 19).

The robust large-scale modern genetic technologies based on sequencing have allowed detailed mapping of even subtle genetic alterations in tumors during the course of disease progression (20). Outstanding genetic heterogeneity within individual primary tumors has been observed in various cancer types (21-23). For example, malignant melanoma is highly heterogeneous at the single lesion level, and different stages of tumor development also differ in their genetic profile (24). It is very likely that this phenomenon is very common in many solid cancer types (25, 26) and blood malignancies (27). The comparative analysis of primary tumor and related metastatic disease brings further evidence that only certain genotypic variants from the source pool are successful in the process of metastasis formation (28, 29). These observations also explain the specific affinity of cancer cells originating from distinct types of primary tumors for specific targets such

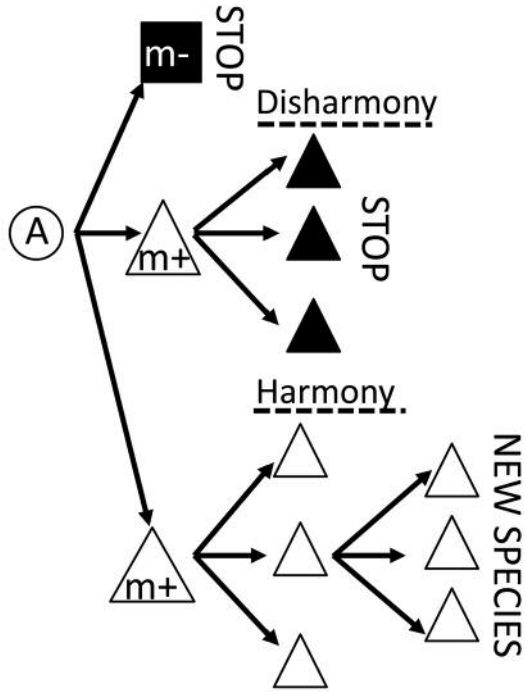

Figure 1. *Mutations are accumulated in cells including germ cells of the ancestor organism (A) over time. When these mutations have a negative effect on the organism (m–), it is usually not viable (STOP). The future of organisms where mutations are beneficial (m+) depends on harmony with other organisms in the ecosystem. Only beneficially mutated organisms coexisting in harmonic balance with the whole ecosystem maintain these mutations resulting in the development of new species from the original ancestor organism.*

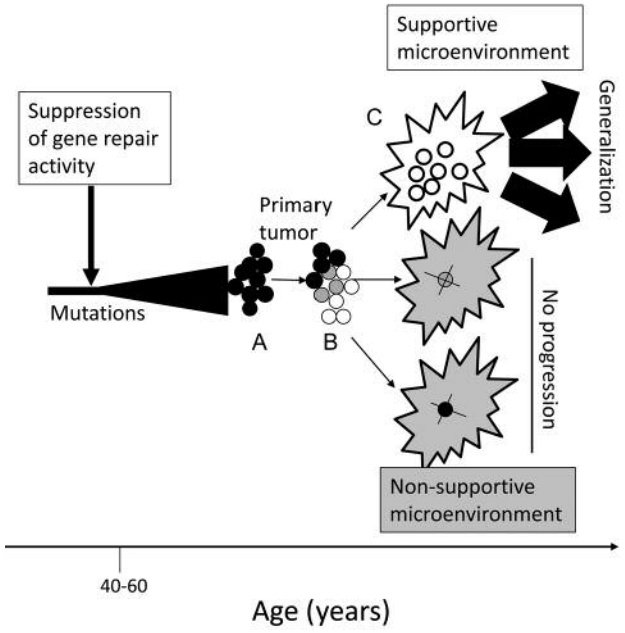

Figure 2. *Gene repair activity is significantly suppressed with increasing age, which leads to formation of primary malignant tumors (A). The genetic heterogeneity of primary tumor increases (B). New mutations are extensively formed during tumor progression leading to the beginning of cancer progression. Interactions within the microenvironment support the growth of cancer cells with a distinct genotype leading to successful tumor evolution and cancer generalization (C). The advanced stages of disease including metastasis can be genetically different from the initial stage of cancer. This development is very similar to the Darwinian development of new species, and the tumor cells with altered genotype forming a balanced ecosystem with a tumor microenvironment are able successfully grow to advanced stages of the disease.*

as the brain, lungs or liver, while other organs/tissues are mostly not affected by tumor circulating/initiating cells (30). The growth of genetic diversity during tumor progression seems to be associated with either positive or negative selection resulting in the establishment of aggressive phenotype with metastatic behavior (31). In addition to mutations, the participation of epigenetic modifications in cancer cell heterogeneity has also been noted (32), but this appears to be a non-Darwinian type of evolutionary approach. Of note, other mechanisms may also participate in cancer evolution since the degree of genetic diversity and number of mutations seem to be too high (thousands) to be explainable only according to Darwin's theory (33).

Darwin's idea of the evolution of macro-organisms as a species (34) tempts us to project this concept to the microscopic landscape of our own tissues in order to explain the course of cancer progression. The genetic changes accumulated in cancer cells, however, do not seem to be fully sufficient to completely drive cancer evolution. Genetically altered cells critically require complicated interactions with their surrounding microenvironment. These direct or indirect interactions generate positive/negative selection pressures applied to cancer cells with new mutations (35) (Figure 2). Such a permissive microenvironment must support the growth of genetically altered cancer cells, including cancer-initiating cells with stem cell properties. Indeed, cancer stem cells as found for example in acute lymphoblastic leukemia (see below) vary significantly in their genotype and phenotype, which make them a clinically elusive target (36). Every therapeutic approach affects this cancer ecosystem and brings a new stimulus, positively or negatively selecting cells to survive or to die. This also clears the ecosystem and enables a better chance for surviving cells to proliferate. This so-called tumor evolution probably represents the crucial reason for the failure of cancer treatment, including of modern targeted therapy.

These genetic alterations are well reflected by the morphological heterogeneity of primary tumors (37). However, pathological examination reflecting heterogeneity of cancer samples as evaluated by conventional histological

slides reflects only a short time period and, thus represents an important limitation to the study of tumor evolution over time. Here, self learning algorithms and digitalized tissue-based diagnostics may help us to improve patient care using automated analysis employing specific algorithms refining the diagnosis and treatment through tailor-made therapies; physicians should be aware about the ability to enhance their work by supporting self-learning (*i.e.* without medical control or interaction) digital tools (38-40).

Roles of Chromosomes in Development of Species and Cancer Clones

It may also be speculated that cancer could evolve as a new species of its own kind. The notion of a chromosomal role in cancer development was postulated by Theodore Boveri over 100 years ago (41). Current progress in molecular genetics and biotechnology such as fluorescent *in situ* hybridization, whole-genome profiling, comparative chromosomal mapping and synthetic chromosome approach, have allowed us a better understanding of the role of chromosomes in cancer evolution (41-44). The arrangement of genetic materials in eukaryotic cells is significantly different from that of prokaryotic organisms in that it reflects the evolution-dependent higher stability of eukaryotic chromosomes and more complex regulation of gene activity (45). However, the failure of the mitotic apparatus or chromosomal structure or function can cause aneuploidy. Although present in normal tissue, aneuploid cells are genetically abnormal and frequently associated with many types of cancer including breast, lung, prostatic, lung and ovarian, and glioblastoma (46-49). Of note, aneuploidy, mainly tetraploidy, frequently observed in cancer also seems to be associated with ageing (50) and also plays a certain role in stem cell biology (51).

The stability of chromosomes is associated with the function of telomeres. Their failure is related to genomic instability and potentially leads to initiation and progression of cancer, as was noted in melanoma, glioblastoma and several types of sarcomas but not yet in tumors of epithelial origin (52, 53). Changes of chromosomal integrity, such as breaks, translocations or deletions, can significantly influence gene expression, including resulting in the formation of fusion proteins that acquire new properties and can participate in cancer initiation and evolution. These chromosomal abnormalities can also influence the epigenetic regulation of cell properties important for tumor biology. This cancer heterogeneity can be detected by cytogenetic inspection of cancer cells (54). In this context, evolution of tumor clones has also been recorded during repeated long-term molecular analyses at a single patient level (55). It is also well known that ribosomal DNA repair is associated with the stability of chromosomes. Accordingly, an inappropriate repair of ribosomal DNA may lead to centromeric, pericentromeric and telomeric instability, with important consequences related to chromosomal aberrations also being responsible for tumor heterogeneity (56).

In particular, aneuploidy is closely related to karyotype instability and most importantly can separate several closely collaborating or co-regulated genes. Most fatal consequences are observed when chromosomal aberrations affect specific locations encoding proteins responsible for synthesizing, segregating and repairing chromosomes. For example, among commonly observed chromosomal aberrations in patients with chronic lymphocytic leukemia, the most unfavorable prognosis is associated with the deletion of 17p chromosome, which results in the loss of *TP53* gene allele (57). Soft-tissue tumors represent a large family from benign to highly malignant lesions. The discrimination between benign/semi-malignant and highly malignant tumors is crucial for selection of appropriate therapy (58). *MET* proto-oncogene (a member of the receptor tyrosine kinase family) overexpressing myxofibrosarcoma is a common highly malignant soft-tissue tumor. This tumor exhibits polysomy of chromosome 7, which causes a high expression of *MET*, leading to an unfavorable prognosis (59). However, as seen during species evolution, only a small minority of mutations acquire reproductive autonomy (54). Other examples demonstrating the role of chromosomes in malignant disease evolution are discussed below.

Cancer as an Ecosystem

The tumor is not an autonomous entity composed entirely of genetically altered cells; it also contains numerous genetically unaltered cell types. Collectively, all these various cell types form a complex ecosystem (60, 61). Cancer-associated fibroblasts (CAFs), immune and endothelial cells, and other elements with their products, including the extracellular matrix, reciprocally interact with malignant cells within the tissue microenvironment and in such a way influence the biological properties of tumors (Figure 3). As space and nutrition are limited, normal and cancer cells, including different cancer clones, complete with each other, resulting in tumor evolution. All these factors include, but are not limited to local aggressiveness and metastasis formation in a cancer type-dependent manner (62, 63). Therefore, targeting the ecosystem/niche rather than the cancer clone alone may have an important clinical implication based on tumor biology. Specific molecules targeting selected steps in angiogenesis, extracellular matrix formation, CAF biology, several signaling pathways, growth factors and the immune system were experimentally considered to be effective in the modulation the tumor microenvironment (TME), thus have been selected for several clinical trials [reviewed by Gál *et al.* (64)].

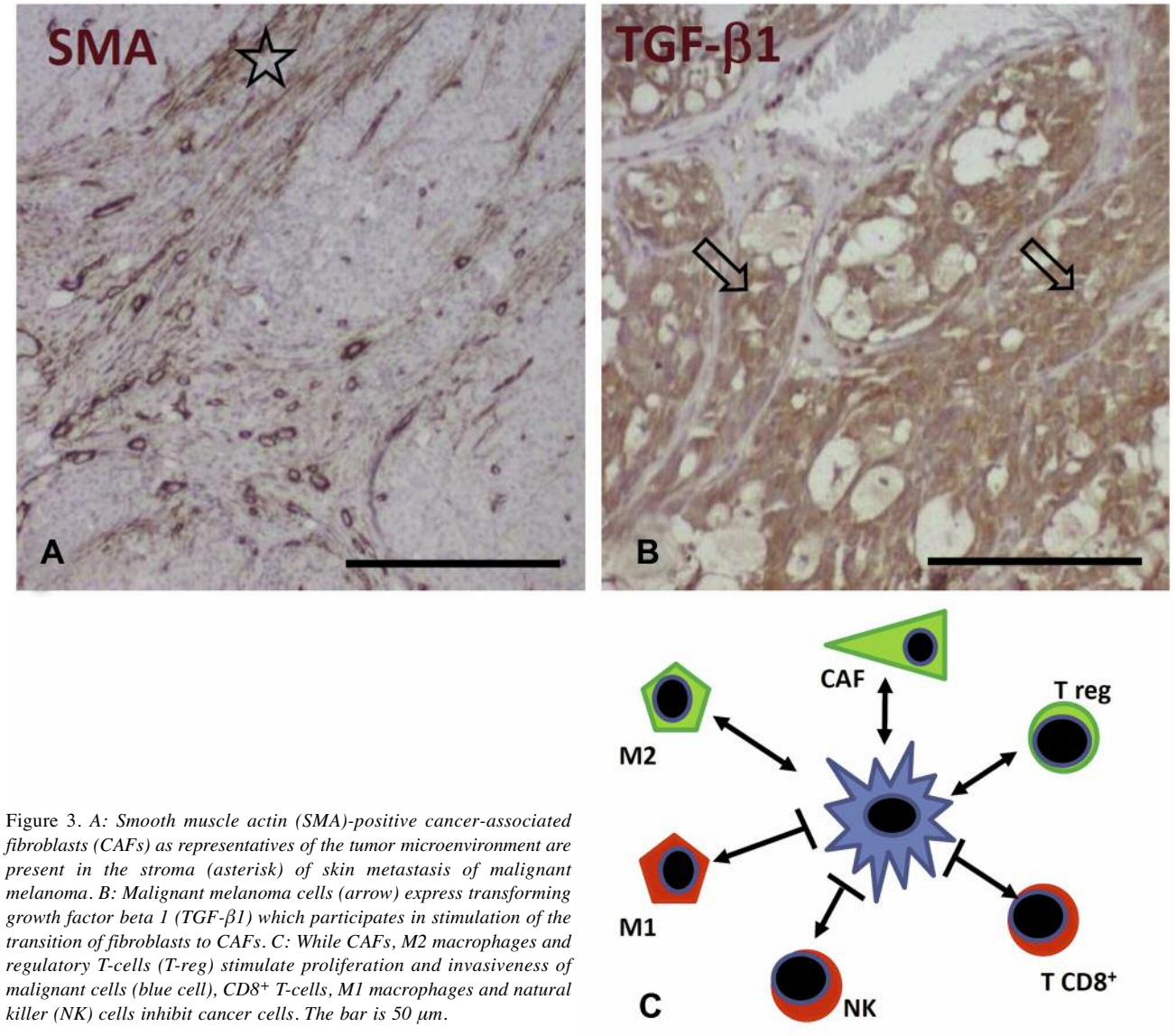

Figure 3. *A: Smooth muscle actin (SMA)-positive cancer-associated fibroblasts (CAFs) as representatives of the tumor microenvironment are present in the stroma (asterisk) of skin metastasis of malignant melanoma. B: Malignant melanoma cells (arrow) express transforming growth factor beta 1 (TGF-β1) which participates in stimulation of the transition of fibroblasts to CAFs. C: While CAFs, M2 macrophages and regulatory T-cells (T-reg) stimulate proliferation and invasiveness of malignant cells (blue cell), CD8+ T-cells, M1 macrophages and natural killer (NK) cells inhibit cancer cells. The bar is 50 μm.*

Based on the knowledge of how cancer and non-cancer adult stem cells interact with the microenvironment to remain undifferentiated, it should not be a surprising finding that the function of the TME is cancer-type unspecific. Accordingly, CAFs isolated from cutaneous squamous or basal cell carcinomas, malignant melanoma as well as breast cancer were found to significantly influence the pattern of differentiation of breast cancer cells; they acquired a more aggressive phenotype (65). Similarly, CAFs isolated from malignant melanoma significantly potentiated the invasiveness of glioblastoma cells in an *in vitro* model (66). This progressive activity of CAFs seems to be dependent on interleukin-6 and -8 signaling (67-70), thus blockade of these pathways, for instance with a novel class of drugs recently referred to as 'migrastatics' (71, 72), bear certain promise in cancer treatment (67, 70). CAFs also produce factors inhibiting anticancer activities of natural killer (NK) cells and cytotoxic $CD8^+$ T-cells, and stimulate tumor-supporting regulatory T-cells and M2 macrophages (73).

On the other hand, cancer cells can, under certain conditions, be recognized and killed by immune cells also representing a significant part of the TME (74). Viviparous individuals, including humans, have developed a mechanism preventing rejection of foreign tissue (embryo/fetus) by suppressing their immune system (75). Hypothetically, such a mechanism might also contribute to the reduced ability to effectively recognize self malignant cells when compared to non-viviparous organisms. Furthermore, the most intensive evolutionary pressure on the development of our own immune system has been mediated by contact with bacteria, parasites and viruses, leaving little time to evolve to fight against cancer (76).

Therefore, the recognition of cancer cells and their consequent clearance by the immune system was recently improved by application of targeted antibodies and by application of whole activated cells (dendritic cells, $CD8^+$ cytotoxic T-cells) during immunotherapy, which represents a recent reasonable clinical option (77, 78). However, the primary lack of effect or later acquired disease resistance to this therapy represents a serious clinical complication frequently resulting in the failure of treatment in individual patients (79).

Carcinomas: Wide Spectrum of Mutations and Aberrations

An understanding of carcinoma development in evolutionary terms is perhaps most effectively described by dividing it into three basic steps. These are initiation of the tumor by a key gene mutation at a single-cell level, followed by clonal expansion into a multicellular neoplasm and its introduction into the ecosystem of the organism. Statistical analysis of numerous cancer types has determined a strong correlation between cancer development, life time and the number of normal stem cell divisions in a healthy tissue (80).

Lung cancer. Non-small-cell lung cancer (NSCLC) represents a very good example of the most genetically diverse cancer types. Here, detection of tyrosine kinase-activating epidermal growth factor receptor (*EGFR*) mutations and anaplastic lymphoma kinase (*ALK*) gene mutations by modern molecular technologies has been well applied in clinical practice to select distinct cohorts of patients for personalized first-line therapy with *EGFR* (gefitinib) and *ALK* (crizotinib) tyrosine-kinase inhibitors, respectively (81, 82). Although these mutations are not present in all carcinomas (83), recent genome-wide screenings have revealed that distinct, but different, genetic abnormalities are typically present in other carcinomas, which provides further opportunities to develop novel personalized molecularly-targeted therapeutic strategies in an attempt to improve the care of specific groups of patients (84, 85).

In addition to several specific gene mutations different for small-cell lung cancer (SCLC) and NSCLC, both types of lung cancer also involve a specific set of common numeric and structural cytogenetic abnormalities. The latter include non-reciprocal translocations and recurrent losses involving chromosomes 1p, 3p, 6q, 9p, 11p, 15p and 17p, representing changes in well known and also potential tumor-suppressing genes (86, 87).

Pancreatic cancer. In the pancreas, familial cancer occurs only approximately 5 years earlier than sporadic disease (88). Pancreatic cancer may be considered as a robust example of Darwinian evolution (89) and is characterized by three stages: (i) initiation of the tumor by the acquisition of a driver gene mutation in a cell of origin, (ii) clonal expansion of the mutation-carrying cell into a multicellular neoplasm, and (iii) introduction of the neoplasm into the TME (90). Among many risk factors (alcohol, smoking, diabetes, obesity, *etc.*) promoting pancreatic cancer development (91), the mechanism by which obesity or type II diabetes induce a chronic pro-inflammatory state and hyperinsulinemia are still poorly understood (92). For example, hyperglycemia supports epithelial–mesenchymal transition and stem cell properties in pancreatic ductal epithelial cells (93). Over 90% of invasive adenocarcinomas of the pancreas harbor oncogenic mutations in Kirsten rat sarcoma virus (*KRAS*) (94). In this context, it has been demonstrated that a high fat diet in association with oncogenic *KRAS* activation increases the formation of pancreatic tumors (95). As well as *KRAS* mutation, cyclin-dependent kinase Inhibitor 2A (*CDKN2A*), *TP53* and SMAD family member 4 (*SMAD4*) are important candidates that may lead to synergistic effects between mutations. Thus, improved understanding of crucial driver genes that create a complex tumorigenic network may lead to the development of new targeted and immune-modifying therapies into current treatment programs (96).

Furthermore, cytogenetic analysis of pancreatic carcinomas have identified alterations in the form of gene rearrangement or losses in following chromosomes, 1p, 3p, 6q, 8p, 12p, 16q, 17d and 18d. Importantly, chromosomes 17 and 18 carry the *TP53* and deleted in colorectal carcinoma (*DCC*) genes, respectively (97). Other examples represent loss of chromosome 20, alterations of chromosome 8 and amplification of *c-MYC* oncogene (98). On the other hand, the allelic loss of a locus at chromosome 3p25, which may contain a novel pancreatic endocrine tumor-suppressor gene, may represent a new potential marker of prognosis (99). Of note, a nondiploid or aneuploid DNA content is usually associated with advanced tumor stage and shorter survival in patients with pancreatic cancer (100). However, to date, the majority of chromosomal-specific changes identified in pancreatic carcinoma are of a poor diagnostic value.

Colon cancer. In contrast to pancreatic cancer, in tissues with much higher proliferation activity, *e.g.* the colon, genetically familial cancer occurs even more than a decade earlier than sporadic cancer (101). In addition to genetic mutations of a single or a group of genes, other relevant mechanism may also exist through evolution of new cell clones that are inherently resistant to anti-neoplastic treatment, by a mechanism called cell fusion (102). During this process two cells combine their plasma membranes and become a single one, possessing and retaining certain genetic information from each parent cell (103). Cell fusion represents a very effective way of rapid phenotypic evolution that gives rise to cells with new properties at a rate that exceeds that of random mutagenesis. For example, in colonic cancer, a number of proteins, namely tetraspanin CD81/CD9, a disintegrin and

metalloproteinase domain-containing protein 10 (ADAM10), GTP-binding protein α13, radixin, myosin regulatory light chain, and Ras homolog gene family, member A (RhoA), were identified to play a role in the regulation of cell fusion. More importantly, fused cells developed resistance to both 5-fluorouracil and oxaliplatin (104).

Colorectal cancer represents one of the most frequent types of malignant lesion with a characteristic spectrum of cytogenetic aberrations. It has been shown that detailed cytogenetical mapping can predict the response of specific tumors to radiation therapy well. Namely alterations of chromosomes 1p, 8p, 17p, 18q, 8q, 13q, and 20q, seem to be clinically relevant (105).

Breast cancer. Breast cancer is another extraordinary example displaying considerable inter- and intra-tumoral genetic heterogeneity. Currently, it is accepted that no two breast cancers are genetically identical, and that both primary and metastatic tumors harbor a diverse set of genomic alterations (106). For example, mutations of estrogen receptor 1 (ER-α, *ESR1*) ligand-binding domain are present at much higher frequencies in metastatic estrogen-positive tumors from patients who have experienced relapse on therapy with aromatase inhibitors (107). These included highly recurrent genetic mutations of p.Tyr537Ser, p.Tyr537Asn and p.Asp538Gly amino acids. Molecular dynamics simulations have revealed that the structures of the Tyr537Ser and Asp538Gly mutants probably involve hydrogen bonding of the mutant amino acids with Asp351. Such change favors the agonist conformation of the receptor, leading to estrogen-induced transcription and proliferation in the absence of hormone, and also reduced efficacy of anti-estrogen treatment.

Chromosomal aberrations, such as chromosome 1q21.3 amplification has been established as a good prognostic marker of cancer recurrence. This aberration affects amplification of S100 calcium-binding protein (S100A) family members, mainly *S100A7*, *S100A8*, and *S100A9*, and IL1 receptor-associated kinase 1, which indicates unfavorable therapy response (108).

Renal carcinomas. Although the complexity of chromosomal aberrations has been comprehensively studied in hematological malignancies, they can be also seen in solid tumors. For example, cytogenetic inspection is very important for diagnosis of chromophobe renal cell carcinoma where loss of seven chromosomes (namely 1, 2, 6, 10, 13, 17, and 21) is typically observed. Furthermore, 31 somatic mutations are being cytogenetically investigated to better refine diagnosis and to improve individualized therapy and prognosis (109). Of note, both methodological approaches are also used for the indication for targeted immunotherapy in renal cell carcinoma (110).

Melanoma: Mutation of B-Raf proto-oncogene, serine/threonine kinase (BRAF) and chromosomal alterations. The BRAF molecule is a member of the signaling cascade transmitting signal from a membrane-bound growth factor receptor associated with tyrosine kinase activity in the nucleus. As a consequence of the signaling, receptor-dependent cell proliferation is initiated. When *BRAF* harbors a set of distinct mutations (*e.g.* $BRAF^{V600E}$), the cascade is activated even without the interaction of growth factor with the receptor (111, 112). Relevant to the context of this article, primary melanoma lesions are heterogeneous and contain both *BRAF*-mutated and wild-type areas (113). Vemurafenib, a small BRAF inhibitor, successfully inhibits the activity of aberrant $BRAF^{V600E}$ in cutaneous malignant melanoma and metastases (114). Unfortunately, after frequently observed initial massive reduction of tumor burden, disease progression is evident in some patients. This phenomenon can be explained by acquired resistance to the therapy (113, 115). The acquisition of resistance in $BRAF^{V600E}$-mutated melanoma cells to small - molecule inhibitors seems to be associated with a high activity of CAFs and their supportive effect on malignant cells (116, 117). Fibroblasts isolated from the nonlesional skin of a patients with $BRAF^{V600E}$ melanoma at the stage of resistance to vemurafenib exhibit great similarity to CAFs at both morphological and molecular levels (118). This suggests a systemic effect of the malignant disease, which can also be observed in distant fibroblasts and may represent a mechanism actively reshaping the normal tissue microenvironment into a cancer-supporting one.

This phenomenon can also be demonstrated in photodamaged skin. Normal dermal fibroblasts from the sun-exposed areas of the body harbor multiple non-tumorigenic genetic mutations in contrary to fibroblasts from sun-protected areas (119). These cells acquire properties similar to CAFs. In such a predisposed microenvironment, the ecosystem is permissive or even supportive of cancer development. Consequently, invasion by malignant clones or docking of circulating malignant cells occurs. This niche also facilitates metastatic behavior and helps cancer cells to endure and acquire resistance to therapy.

Numerous cytogenetical changes were also detected in malignant melanoma that contrasts with negligible chromosomal alteration in benign melanocyte lesions, where amplification of 4q12 chromosome, where the genes for (platelet-derived growth factor receptor alpha (*PDGFRA*) and for KIT (a member of the type III transmembrane receptor tyrosine kinase family) are located (120).

Acute leukemia: Fusion of ETS variant 6 (ETV6)/Runt-related transcription factor 1 (RUNX1), BCR (B-cell receptor), RhoGEF and GTPase activating protein (BCR)–proto-oncogene 1, non-receptor tyrosine kinase [Abelson murine leukemia viral oncogene homolog 1 (ABL1)] mutation and chromosomal aberrations. The evolution

model posited by Charles Darwin may also be implicated in the most frequently occurring childhood malignancy, acute lymphoblastic leukemia (ALL). Firstly, it was suggested that this cancer would be easier to study due to its quicker development and genetic simplicity when compared for instance to a carcinoma (15). Based on published data it is logical to suggest that in many types of cancer, patients actually suffer from several independent tumors derived from one clone (121). Therefore, personalized treatment becomes trickier, since prior to and during the initial therapy, new clones may develop that may significantly differ in genetic aberrations. ALL represents a good example onf the important role of the initiating progenitor cells with stem cell potential, in which *ETV6–RUNX1* gene fusion is expected to be an early or initiating genetic lesion followed by a modest number of copy number alterations (122). These are independently and reiteratively acquired in tumor subclones of individual patients. Some mutations are neutral or passenger, but in particular, mutations involving *ETV6*, paired box protein 5 (*PAX5*) and *CDKN2A* (*p16*) are highly recurrent and may be considered as functional mutations since they encode protein with functions relevant to leukemogenesis (123-125). Furthermore, a monozygotic twin study revealed that after *ETV6–RUNX1* fusion, all subsequent postnatal mutations were probably secondary functional mutations which arose as a result of disease evolution (126).

On the other hand, the cancer stem cells of chronic myeloid leukemia, in particular during the chronic phase, are driven by a single fused *BCR–ABL1* gene with only little genetic variability (127). Therefore, treatment with imatinib, a specific tyrosine kinase inhibitor of ABL, c-KIT and PDGFR, has high efficiency during the past almost two decades (128). Thus, cancer heterogeneity, which fosters disease evolution, is a key factor responsible for the release of resistant subclones, and clearly correlates with the efficiency of currently used treatment.

The important role of chromosomes in tumorigenesis, subclone evolution and disease progression over time can also be well shown in acute myeloid leukemia (AML) (129). A highly specific subset of mutations common for *de novo* AML was identified, while a distinct subset of mutations commonly found in myelodysplastic syndrome progressing to AML was identified in secondary AML (130, 131). Somatic mutation studies in AML clonal evolution have identified key early leukemogenesis-driving genes namely DNA (cytosine-5)-methyltransferase 3A (*DNMT3A*), putative polycomb group protein (*ASXL1*) and tet methylcytosine dioxygenase 2 (*TET2*) in leukemia stem cells and progenitor cells (132-134). Recurrent mutations in these genes together with mutations of splicing factor 3B subunit 1 (*SF3B1*) and splicing factor, arginine/serine-rich 2 (*SRSF2*) were associated with clonal hematopoietic expansion in the healthy elderly (135, 136). Based on preliminary data, it was speculated that analogously to monoclonal gammopathy of undetermined significance progressing to multiple myeloma, the aforementioned clonal hematopoietic expansion may progress to hematological malignancy at a very similar rate (129). The importance of chromosomal aberrations for precise AML classification is indisputable, as can be seen in the World Health Organization classification of myeloid neoplasms, which is predominantly based upon chromosomal maturations (137). Moreover, sequencing in large cohort patients with AML revealed gene patterns by which it was possible to separate patients into 11 groups, each with its unique clinical importance, prognosis and outcome (138).

Myeloma: Clonal evolution and tumor microenvironment. Myeloma, sometimes referred as multiple myeloma (MM), is a blood cancer of fully differentiated plasma cells. Plasma cell malignancies present an ideal model to show how firstly genetic mutations drive tumor progression, followed by the TME. Plasma cells arise from B-cells, which upon proper stimulation in the germinal center become immature plasma cells (139). Sequencing of immunoglobulin heavy-chain variable region of myeloma cells revealed that the first tumorigenic mutations emerge in the germinal center, where two critical mutation-prone processes called isotype class switching and somatic hypermutation take place (140). However, these mutations including multiple trisomies were observed not only in patients with MM, but also in patients with monoclonal gammopathy of undetermined significance and smoldering multiple myeloma (141). These dyscrasias precede practically every MM case, thus it was suggested that these genetic mutations are necessary but not sufficient to drive myeloma transformation (142).

Sequencing studies in MM identified huge genetic heterogeneity of myeloma subclones at diagnosis and five mutated genes of likely pathogenetic significance, specifically *BRAF, KRAS, NRAS* proto-oncogene, GTPase (*NRAS*), *TP53* and family with sequence similarity 46, member C (*FAM46C*) (143-146). What is more is that other studies showed that these mutations are acquired in a non-linear fashion, which is typical of a complex ecosystem of clones competing for survival (147, 148). In myeloma, such an ecosystem is represented by the bone marrow, where a supporting TME responsible for maintenance and evolution of malignant subclones is present (142). Thus, new therapeutic strategies are required to target such a huge body-spread reservoir of malignant subclones.

Tumor and Wound Microenvironments: Do They Express Evolutionary Similarities with Implications in Treatment?

Organisms throughout the whole evolutionary tree differ markedly in their ability to regenerate tissues and organs. While for example, salamanders can regenerate a range of

body parts throughout all stages of life, the regenerative capability of frogs is restricted only to early stages of their development (149). Similarly, mammalian adult skin wound healing results in scar formation, but a fetus has the ability to regenerate and restore the original architecture and function of *dermis* (150). Recent studies have shown that reduced inflammatory reaction in fetal wounds contributes to this scarless healing (151). On the other hand, it is well know that formation of keloids and hypertrophic scars is characterized mainly for humans and results from chronic inflammation in the reticular *dermis* (152). In this context, it is interesting that some similarities between tumors and pathological scars have been noted (153), including the positive role of an inflammation-supporting micromilieu (154) resulting in overproduction of immature collagen (155).

Growth factors from the transforming growth factor beta (TGFβ) family also have a characteristic expression pattern in hypertrophic compared to scarless healing (156). Whereas fetal healing is characterized by up-regulation of TGFβ3 and down-regulation of TGFβ1 and -β2, hypertrophic skin repair also results from an inverse expression pattern of these TGFβ molecules. Accordingly, prolonged presence of activated α-smooth muscle-expressing fibroblast, so-called myofibroblasts, can frequently be seen in hypertrophic scars but only rarely in keloids (157, 158). Myofibroblasts play very important functional roles in wound contraction and substantially modulate biological properties of tumors (62, 64, 159-162). For example, using a breast tumor xenograft model, it was shown that resident human mammary fibroblasts were converted into cancer-promoting myofibroblasts during the course of disease progression (163). These CAFs acquired two autocrine signaling loops, mediated by TGFβ and stromal cell-derived factor 1 (SDF1) cytokines, thus with remarkable similarities to myofibroblast generation from local dermal fibroblasts in healing wounds induced by TGFβ1 (164).

Moreover, hypertrophic scars and keloids are also considered benign tumors, thus they are in some cases treated with chemotherapeutic drugs (165). Interestingly, these cells also acquired a certain level of drug resistance that is characteristic of malignant tumors. Scar fibroblasts showed stronger resistance to both verapamil and etoposide than normal fibroblasts. Furthermore, scar fibroblasts also expressed more P-glycoprotein and multidrug resistance-associated protein 1 (MRP1) than their normal counterparts (166). However, down-regulation of the expression of drug transporters or disrupting the drug transporter–actin filament interaction was effective in reducing their resistance to chemotherapy.

In the context of this article and evolution theory, it is necessary to note that multiple *in vitro* and *in vivo* studies revealed that there is a close correlation between regeneration and generation of tumors. Studies comparing tissue repair/regeneration with aspects of malignancy on the molecular level revealed that these two processes do have even more in common (70, 167). In particular, the proliferative phase of wound repair is characterized by the production of granulation tissue whose architecture is very similar to that of tumor stroma. Here, fibroblasts produce several cytokines/chemokines (*e.g.* IL6, IL8, CXCL1), growth/adhesion regulatory galectins (*e.g.* GAL1) and growth factors [*e.g.* vascular endothelial growth factor (VEGF), TGFβ, and epidermal growth factor (EGF)] that on the one hand stimulate angiogenesis, and on the other hand support the process of re-epithelization and maintain proliferation of poorly differentiated epithelial cells (168-171). Therefore, these data also suggest that cancer utilizes phylogenetically older pathways reflected in the similarity of both wound and tumor microenvironments.

Conclusion

Although, clinically available morphological, cytogenetic and genome-wide expression analysis are available to monitor the evolution of several cancer types at a single patient level, application of the evolutionary theory may provide new philosophical inspiration for further development of personalized cancer treatment (172, 173). Malignant genetically altered cancer cells together with the TME form a functional ecosystem promoting the growth and spreading of tumors. Therefore, targeting the TME rather than the cancer cell itself may have an important clinical implication in stopping tumor evolution in terms of acquisition of resistance to the applied therapy or to the formation of distant metastases and thus be the hallmark of personalized cancer therapy (174). Sophisticated mathematical models of cancer progression support the evolutionary concept that provides a better understanding of the process of tumor development and progression with all clinically relevant consequences (175). Of note, the importance of cell fusion in cancer progression and resulting chemoresistance in metastatic colonic cancer has also been well demonstrated (104). Hence, specific selection pressure applied to the cancer cells, including cancer-initiating (progenitor/stem) cells, may influence therapy in the near future. If so, novel anticancer treatments might effectively target even resistant cancer cells (176).

Based on the presented data, it is not surprising that chromosomal abnormalities, including numerical ones, also participate in tumor initiation and intratumoral heterogeneity, resulting in the evolution of tumors and metastasis formation during disease progression. It explains the complexity and speed of metabolic and functional changes typical of cancer (177). Detailed analysis of malignant diseases on the chromosomal level strongly supports the participation of Darwinian and also Lamarckian principles in disease evolution (15). The prevailing view that the evolution of cancer cells

only follows Darwinian selection has never been rigorously tested in all types of cancer. For example, a study of hepatocellular carcinoma agreed well with the non-Darwinian model, with no evidence of positive Darwinian selection (33). Therefore, the question whether tumor evolution is dominantly driven by Darwinian or non-Darwinian selection remains open. A better understanding of all types of mutations and their role in tumor evolution can help refine diagnostics and open new horizons for cancer therapy.

Conflicts of Interest

The Authors declare no conflict of interest exists in regard to this study.

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References

1 Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, Maclntyre MF, Allen C, Hansen G, Woodbrook R, Wolfe C, Hamadeh RR, Moore A, Werdecker A, Gessner BD, Te Ao B, McMahon B, Karimkhani C, Yu CH, Cooke GS, Schwebel DC, Carpenter DO, Pereira DM, Nash D, Kazi DS, De Leo D, Plass D, Ukwaja KN, Thurston GD, Jin KY, Simard EP, Mills E, Park EK, Catala-Lopez F, DeVeber G, Gotay C, Khan G, Hosgood HD, Santos IS, Leasher JL, Singh J, Leigh J, Jonas JB, Sanabria J, Beardsley J, Jacobsen KH, Takahashi K, Franklin RC, Ronfani L, Montico M, Naldi L, Tonelli M, Geleijnse J, Petzold M, Shrime MG, Younis M, Yonemoto N, Breitborde N, Yip P, Pourmalek F, Lotufo PA, Esteghamati A, Hankey GJ, Ali R, Lunevicius R, Malekzadeh R, Dellavalle R, Weintraub R, Lucas R, Hay R, Rojas-Rueda D, Westerman R, Sepanlou SG, Nolte S, Patten S, Weichenthal S, Abera SF, Fereshtehnejad SM, Shiue I, Driscoll T, Vasankari T, Alsharif U, Rahimi-Movaghar V, Vlassov VV, Marcenes WS, Mekonnen W, Melaku YA, Yano Y, Artaman A, Campos I, MacLachlan J, Mueller U, Kim D, Trillini M, Eshrati B, Williams HC, Shibuya K, Dandona R, Murthy K, Cowie B, Amare AT, Antonio CA, Castaneda-Orjuela C, van Gool CH, Violante F, Oh IH, Deribe K, Soreide K, Knibbs L, Kereselidze M, Green M, Cardenas R, Roy N, Tillmann T, Li YM, Krueger H, Monasta L, Dey S, Sheikhbahaei S, Hafezi-Nejad N, Kumar GA, Sreeramareddy CT, Dandona L, Wang HD, Vollset SE, Mokdad A, Salomon JA, Lozano R, Vos T, Forouzanfar M, Lopez A, Murray C, Naghavi M and Dis GB: The global burden of cancer 2013 global burden of disease cancer collaboration. JAMA Oncol *1*: 505-527, 2015.

2 Hanahan D: Rethinking the war on cancer. Lancet *383*: 558-563, 2014.

3 Lowy DR and Collins FS: Aiming high–changing the trajectory for cancer. N Engl J Med *374*: 1901-1904, 2016.

4 On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. Br Foreign Med Chir Rev *25*: 367-404, 1860.

5 Smetana K, Lacina L, Szabo P, Dvorankova B, Broz P and Sedo A: Ageing as an important risk factor for cancer. Anticancer Res *36*: 5009-5017, 2016.

6 Datta RS, Gutteridge A, Swanton C, Maley CC and Graham TA: Modelling the evolution of genetic instability during tumour progression. Evolut Applications *6*: 20-33, 2013.

7 Lodato MA, Rodin RE, Bohrson CL, Coulter ME, Barton AR, Kwon M, Sherman MA, Vitzthum CM, Luquette LJ, Yandava CN, Yang PW, Chittenden TW, Hatem NE, Ryu SC, Woodworth MB, Park PJ and Walsh CA: Aging and neurodegeneration are associated with increased mutations in single human neurons. Science *359*: 555-558, 2018.

8 Smetana K, Dvořánková B, Lacina L, Szabo P, Brož P and Šedo A: Cancer, the price for longevity. *In*: Aging: Exploring a complex phenomenon. CRC Press, pp. 237-245, 2018.

9 Pinto da Costa J: A synopsis on aging. *In*: Aging: Exploring a complex phenomenon. CRC Press, pp. 3-22, 2018.

10 Wang X, Zhang H, Su L and Lv Z: The genetic program of aging. *In*: Aging: Exploring a complex phenomenon. CRC Press, pp. 117-134, 2018.

11 Darwin CR: On the origin of species by meand of natural selection , or the preservation of favoured races in the strungle for life. John Murray, London, 1859.

12 Hakem R: DNA-damage repair; the good, the bad, and the ugly. EMBO J *27*: 589-605, 2008.

13 Podos J and Nowicki S: Beaks, adaptation, and vocal evolution in darwin's finches. Bioscience *54*: 501-510, 2004.

14 Sato A, Tichy H, O'hUigin C, Grant PR, Grant BR and Klein J: On the origin of Darwin's finches. Mol Biol Evol *18*: 299-311, 2001.

15 Greaves M: Darwin and evolutionary tales in leukemia. The Ham-Wasserman lecture. Hematology Am Soc Hematol Educ Program *2009*: 3-12, 2009.

16 Grove CS and Vassiliou GS: Acute myeloid leukaemia: A paradigm for the clonal evolution of cancer? Dis Model Mech *7*: 941-951, 2014.

17 Wang E, Voiculescu S, Le Poole IC, El-Gamil M, Li X, Sabatino M, Robbins PF, Nickoloff BJ and Marincola FM: Clonal persistence and evolution during a decade of recurrent melanoma. J Invest Dermatol *126*: 1372-1377, 2006.

18 Arozarena I and Wellbrock C: Overcoming resistance to BRAF inhibitors. Ann Transl Med *5*: 387, 2017.

19 Brady SW, McQuerry JA, Qiao Y, Piccolo SR, Shrestha G, Jenkins DF, Layer RM, Pedersen BS, Miller RH, Esch A, Selitsky SR, Parker JS, Anderson LA, Dalley BK, Factor RE, Reddy CB, Boltax JP, Li DY, Moos PJ, Gray JW, Heiser LM, Buys SS, Cohen AL, Johnson WE, Quinlan AR, Marth G, Werner TL and Bild AH: Combating subclonal evolution of resistant cancer phenotypes. Nat Commun *8*: 1231, 2017.

20 Macaulay IC, Teng MJ, Haerty W, Kumar P, Ponting CP and Voet T: Separation and parallel sequencing of the genomes and transcriptomes of single cells using g&t-seq. Nat Prot *11*: 36-58, 2016.

21 Erramuzpe A, Cortes JM and Lopez JI: Multisite tumor sampling enhances the detection of intratumor heterogeneity at all different temporal stages of tumor evolution. Virchows Arch *472*: 187-194, 2018.

22 Orzan F, De Bacco F, Crisafulli G, Pellegatta S, Mussolin B, Siravegna G, D'Ambrosio A, Comoglio PM, Finocchiaro G and Boccaccio C: Genetic evolution of glioblastoma stem-like cells from primary to recurrent tumor. Stem Cells *35*: 2218-2228, 2017.

23 Pelosi E, Castelli G and Testa U: Pancreatic cancer: Molecular characterization, clonal evolution and cancer stem cells. Biomedicines *5*: 65, 2017.

24 Shain AH, Yeh I, Kovalyshyn I, Sriharan A, Talevich E, Gagnon A, Dummer R, North J, Pincus L, Ruben B, Rickaby W, D'Arrigo C, Robson A and Bastian BC: The genetic evolution of melanoma from precursor lesions. N Engl J Med *373*: 1926-1936, 2015.

25 Gerlinger M, McGranahan N, Dewhurst SM, Burrell RA, Tomlinson I and Swanton C: Cancer: Evolution within a lifetime. Anal Rev of Genet *48*: 215-236, 2014.

26 Maley CC, Aktipis A, Graham TA, Sottoriva A, Boddy AM, Janiszewska M, Silva AS, Gerlinger M, Yuan YY, Pienta KJ, Anderson KS, Gatenby R, Swanton C, Posada D, Wu CI, Schiffman JD, Hwang ES, Polyak K, Anderson ARA, Brown JS, Greaves M and Shibata D: Classifying the evolutionary and ecological features of neoplasms. Nat Rev Cancer *17*: 605-619, 2017.

27 Varela I, Menendez P and Sanjuan-Pla A: Intratumoral heterogeneity and clonal evolution in blood malignancies and solid tumors. Oncotarget *8*: 66742-66746, 2017.

28 Harbst K, Lauss M, Cirenajwis H, Winter C, Howlin J, Torngren T, Kvist A, Nodin B, Olsson E, Hakkinen J, Jirstrom K, Staaf J, Lundgren L, Olsson H, Ingvar C, Gruvberger-Saal SK, Saal LH and Jonsson G: Molecular and genetic diversity in the metastatic process of melanoma. J Pathol *233*: 39-50, 2014.

29 Yates LR, Knappskog S, Wedge D, Farmery JHR, Gonzalez S, Martincorena I, Alexandrov LB, Van Loo P, Haugland HK, Lilleng PK, Gundem G, Gerstung M, Pappaemmanuil E, Gazinska P, Bhosle SG, Jones D, Raine K, Mudie L, Latimer C, Sawyer E, Desmedt C, Sotiriou C, Stratton MR, Sieuwerts AM, Lynch AG, Martens JW, Richardson AL, Tutt A, Lonning PE and Campbell PJ: Genomic evolution of breast cancer metastasis and relapse. Cancer Cell *32*: 169-184, 2017.

30 Saunus JM, Quinn MCJ, Patch AM, Pearson JV, Bailey PJ, Nones K, Reed AEM, Miller D, Wilson PJ, Al-Ejeh F, Mariasegaram M, Lau Q, Withers T, Jeffree RL, Reid LE, Da Silva L, Matsika A, Niland CM, Cummings MC, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Wani S, Anderson MJ, Fink JL, Holmes O, Kazakoff S, Leonard C, Newell F, Taylor D, Waddell N, Wood S, Xu QY, Kassahn KS, Narayanan V, Taib NA, Teo SH, Chow YP, Jat PS, Brandner S, Flanagan AM, Khanna KK, Chenevix-Trench G, Grimmond SM, Simpson PT, Waddell N, Lakhani SR and KConFab: Integrated genomic and transcriptomic analysis of human brain metastases identifies alterations of potential clinical significance. J Pathol *237*: 363-378, 2015.

31 Hurst LD and Batada NN: Depletion of somatic mutations in splicing-associated sequences in cancer genomes. Genome Biol *18*: 213, 2017.

32 Mazor T, Pankov A, Song JS and Costello JF: Intratumoral heterogeneity of the epigenome. Cancer Cell *29*: 440-451, 2016.

33 Ling SP, Hu Z, Yang ZY, Yang F, Li YW, Lin P, Chen K, Dong LL, Cao LH, Tao Y, Hao LT, Chen QJ, Gong Q, Wu DF, Li WJ, Zhao WM, Tian XY, Hao CY, Hungate EA, Catenacci DVT, Hudson RR, Li WH, Lu XM and Wu CI: Extremely high genetic diversity in a single tumor points to prevalence of non-darwinian cell evolution. Proc Natl Acad Sci USA *112*: E6496-E6505, 2015.

34 Koonin EV: Towards a postmodern synthesis of evolutionary biology. Cell Cycle *8*: 799-800, 2009.

35 Fortunato A, Boddy A, Mallo D, Aktipis A, Maley CC and Pepper JW: Natural selection in cancer biology: From molecular snowflakes to trait hallmarks. Cold Spring Harb Perspect Med *7*: a029652, 2017.

36 Greaves M: Cancer stem cells: Back to Darwin? Semin Cancer Biol *20*: 65-70, 2010.

37 Vuletić F, Zajec V, Vuletić LB and Seiwerth S: Intratumoral heterogeneity. Diagn Pathol *4*: 257, 2018.

38 Kayser K, Borkenfeld S, Carvalho R, Djenouni A and Kayser G: How to analyze structure and function in tissue – based diagnosis? Diagn Pathol *2*: 106, 2016.

39 Kayser K, Borkenfeld S, Carvalho R and Kayser G: Structure, function, and predictive diagnosis algorithms. Diagn Pathol *1*: 153, 2016.

40 Görtler J, Kayser K, Borkenfeld S, Carvalho R and Kayser G: Cognitive algorithms and digitized tissue – based diagnosis. Diagn Pathol *3*: 248, 2017.

41 Hansford S and Huntsman DG: Boveri at 100: Theodor boveri and genetic predisposition to cancer. J Pathol *234*: 142-145, 2014.

42 Le Scouarnec S and Gribble SM: Characterising chromosome rearrangements: Recent technical advances in molecular cytogenetics. Heredity *108*: 75-85, 2012.

43 Omeir R, Thomas R, Teferedegne B, Williams C, Foseh G, Macauley J, Brinster L, Beren J, Peden K, Breen M and Lewis AM Jr.: A novel canine kidney cell line model for the evaluation of neoplastic development: Karyotype evolution associated with spontaneous immortalization and tumorigenicity. Chromosome Res *23*: 663-680, 2015.

44 Kugoh H, Ohira T and Oshimura M: Studies of tumor suppressor genes via chromosome engineering. Cancers *8*: 4, 2015.

45 Willbanks A, Leary M, Greenshields M, Tyminski C, Heerboth S, Lapinska K, Haskins K and Sarkar S: The evolution of epigenetics: From prokaryotes to humans and its biological consequences. Genet Epigenet *8*: 25-36, 2016.

46 Fang X and Zhang P: Aneuploidy and tumorigenesis. Semin Cell Dev Biol *22*: 595-601, 2011.

47 Simonetti G, Bruno S, Padella A, Tenti E and Martinelli G: Aneuploidy: Cancer strength or vulnerability? Int J Cancer, doi.org/10.1002/ijc.31718, 2018.

48 Edwards PA: Fusion genes and chromosome translocations in the common epithelial cancers. J Pathol *220*: 244-254, 2010.

49 Earp MA, Raghavan R, Li Q, Dai J, Winham SJ, Cunningham JM, Natanzon Y, Kalli KR, Hou X, Weroha SJ, Haluska P, Lawrenson K, Gayther SA, Wang C, Goode EL and Fridley BL: Characterization of fusion genes in common and rare epithelial ovarian cancer histologic subtypes. Oncotarget *8*: 46891-46899, 2017.

50 Tanaka K, Goto H, Nishimura Y, Kasahara K, Mizoguchi A and Inagaki M: Tetraploidy in cancer and its possible link to aging. Cancer Sci *109*: 2632-2640, 2018.

51 Brown A and Geiger H: Chromosome integrity checkpoints in stem and progenitor cells: Transitions upon differentiation, pathogenesis, and aging. Cell Mol Life Sci *75*: 3771-3779, 2018.

52 Bernal A and Tusell L: Telomeres: Implications for cancer development. Int J Mol Sci *19*: 294, 2018.

53 Cleal K, Norris K and Baird D: Telomere length dynamics and the evolution of cancer genome architecture. Int J Mol Sci *19*: 482, 2018.

54 Duesberg P, Mandrioli D, McCormack A and Nicholson JM: Is carcinogenesis a form of speciation? Cell Cycle *10*: 2100-2114, 2011.

55 Meyer M, Reimand J, Lan X, Head R, Zhu X, Kushida M, Bayani J, Pressey JC, Lionel AC, Clarke ID, Cusimano M, Squire JA, Scherer SW, Bernstein M, Woodin MA, Bader GD and Dirks PB: Single cell-derived clonal analysis of human glioblastoma links functional and genomic heterogeneity. Proc Natl Acad Sci USA *112*: 851-856, 2015.

56 Lindstrom MS, Jurada D, Bursac S, Orsolic I, Bartek J and Volarevic S: Nucleolus as an emerging hub in maintenance of genome stability and cancer pathogenesis. Oncogene *37*: 2351-2366, 2018.

57 Hallek M: Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. Am J Hematol *92*: 946-965, 2017.

58 Nishio J: Updates on the cytogenetics and molecular cytogenetics of benign and intermediate soft tissue tumors. Oncol Lett *5*: 12-18, 2013.

59 Ma S, Fan L, Liu Y, Wang Y, Yu K, Wang L, Fang N, Liu F, Guo S and Wang Z: Met-overexpressing myxofibrosarcoma frequently exhibit polysomy of chromosome 7 but not met amplification, especially in high-grade cases: Clinical and pathological review of 30 myxofibrosarcoma cases. Diagn Pathol *13*: 56, 2018.

60 Daoust SP, Fahrig L, Martin AE and Thomas F: From forest and agro-ecosystems to the microecosystems of the human body: What can landscape ecology tell us about tumor growth, metastasis, and treatment options? Evol Appl *6*: 82-91, 2013.

61 Kareva I: What can ecology teach us about cancer? Transl Oncol *4*: 266-270, 2011.

62 Lacina L, Plzak J, Kodet O, Szabo P, Chovanec M, Dvorankova B and Smetana K: Cancer microenvironment: What can we learn from the stem cell niche. Intl J Mol Sci *16*: 24094-24110, 2015.

63 Zivicova V, Gal P, Mifkova A, Novak S, Kaltner H, Kolar M, Strnad H, Sachova J, Hradilova M, Chovanec M, Gabius HJ, Smetana K and Fik Z: Detection of distinct changes in gene-expression profiles in specimens of tumors and transition zones of tenascin-positive/-negative head and neck squamous cell carcinoma. Anticancer Res *38*: 1279-1290, 2018.

64 Gal P, Varinska L, Faber L, Novak S, Szabo P, Mitrengova P, Mirossay A, Mucaji P and Smetana K: How signaling molecules regulate tumor microenvironment: Parallels to wound repair. Molecules *22*: 1818 , 2017.

65 Dvorankova B, Szabo P, Lacina L, Kodet O, Matouskova E and Smetana K: Fibroblasts prepared from different types of malignant tumors stimulate expression of luminal marker keratin 8 in the EM-G3 Breast cancer cell line. Histochem Cell Biol *137*: 679-685, 2012.

66 Trylcova J, Busek P, Smetana K, Balaziova E, Dvorankova B, Mifkova A and Sedo A: Effect of cancer-associated fibroblasts on the migration of glioma cells *in vitro*. Tumour Biol *36*: 5873-5879, 2015.

67 Jayatilaka H, Tyle P, Chen JJ, Kwak M, Ju J, Kim HJ, Lee JSH, Wu PH, Gilkes DM, Fan R and Wirtz D: Synergistic IL-6 and IL-8 paracrine signalling pathway infers a strategy to inhibit tumour cell migration. Nat Commun *8*: 15584, 2017.

68 Jobe NP, Rosel D, Dvorankova B, Kodet O, Lacina L, Mateu R, Smetana K and Brabek J: Simultaneous blocking of il-6 and il-8 is sufficient to fully inhibit caf-induced human melanoma cell invasiveness. Histochem Cell Biol *146*: 205-217, 2016.

69 Jobe NP, Zivicova V, Mifkova A, Rosel D, Dvorankova B, Kodet O, Strnad H, Kolar M, Sedo A, Smetana K, Strnadova K, Brabek J and Lacina L: Fibroblasts potentiate melanoma cells *in vitro* invasiveness induced by uv-irradiated keratinocytes. Histochem Cell Biol *149*: 503-516, 2018.

70 Kolar M, Szabo P, Dvorankova B, Lacina L, Gabius HJ, Strnad H, Sachova J, Vlcek C, Plzak J, Chovanec M, Cada Z, Betka J, Fik Z, Paces J, Kovarova H, Motlik J, Jarkovska K and Smetana K: Upregulation of IL-6, IL-8 and CXCL-1 production in dermal fibroblasts by normal/malignant epithelial cells *in vitro*: Immunohistochemical and transcriptomic analyses. Biol Cell *104*: 738-751, 2012.

71 Gandalovicova A, Rosel D, Fernandes M, Vesely P, Heneberg P, Cermak V, Petruzelka L, Kumar S, Sanz-Moreno V and Brabek J: Migrastatics-anti-metastatic and anti-invasion drugs: Promises and challenges. Trends Cancer *3*: 391-406, 2017.

72 Lacina L, Brabek J, Kral V, Kodet O and Smetana K Jr.: Interleukin-6: A molecule with complex biological impact in cancer. Histol Histopathol *4*: 18033, 2018.

73 Lacina L, Kodet O, Dvorankova B, Szabo P and Smetana K: Ecology of melanoma cell. Histol Histopathol *33*: 247-254, 2018.

74 Chen DS and Mellman I: Elements of cancer immunity and the cancer-immune set point. Nature *541*: 321-330, 2017.

75 Warning JC, McCracken SA and Morris JM: A balancing act: Mechanisms by which the fetus avoids rejection by the maternal immune system. Reproduction *141*: 715-724, 2011.

76 Kaufman J: Evolution and immunity. Immunology *130*: 459-462, 2010.

77 Golubovskaya V, Berahovich R, Zhou H, Xu S, Harto H, Li L, Chao CC, Mao MM and Wu LJ: CD47-CAR-T-cells effectively kill target cancer cells and block pancreatic tumor growth. Cancers *9*: 139, 2017.

78 Rainone V, Martelli C, Ottobrini L, Biasin M, Texido G, Degrassi A, Borelli M, Lucignani G, Trabattoni D and Clerici M: Immunological characterization of whole tumour lysate-loaded dendritic cells for cancer immunotherapy. PLoS One *11*: e0146622, 2016.

79 Bonavida B and Chouaib S: Resistance to anticancer immunity in cancer patients: Potential strategies to reverse resistance. Ann Oncol *28*: 457-467, 2017.

80 Tomasetti C and Vogelstein B: Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science *347*: 78-81, 2015.

81 Inoue A, Kobayashi K, Usui K, Maemondo M, Okinaga S, Mikami I, Ando M, Yamazaki K, Saijo Y, Gemma A, Miyazawa

H, Tanaka T, Ikebuchi K, Nukiwa T, Morita S and Hagiwara K: First-line gefitinib for patients with advanced non-small-cell lung cancer harboring epidermal growth factor receptor mutations without indication for chemotherapy. J Clin Oncol *27*: 1394-1400, 2009.

82 Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S, Nukiwa T and North-East Japan Study G: Gefitinib or chemotherapy for non-small-cell lung cancer with mutated *EGFR*. N Engl J Med *362*: 2380-2388, 2010.

83 Keedy VL, Temin S, Somerfield MR, Beasley MB, Johnson DH, McShane LM, Milton DT, Strawn JR, Wakelee HA and Giaccone G: American society of clinical oncology provisional clinical opinion: Epidermal growth factor receptor (*EGFR*) mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. J Clin Oncol *29*: 2121-2127, 2011.

84 Li TH, Kung HJ, Mack PC and Gandara DR: Genotyping and genomic profiling of non-small-cell lung cancer: Implications for current and future therapies. J Clin Oncol *31*: 1039-1049, 2013.

85 Pao W and Girard N: New driver mutations in non-small-cell lung cancer. Lancet Oncol *12*: 175-180, 2011.

86 Miura I, Graziano SL, Cheng JQ, Doyle LA and Testa JR: Chromosome alterations in human small cell lung cancer: Frequent involvement of 5q. Cancer Res *52*: 1322-1328, 1992.

87 Testa JR and Siegfried JM: Chromosome abnormalities in human non-small cell lung cancer. Cancer Res *52*: 2702-2706, 1992.

88 Petersen GM: Familial pancreatic adenocarcinoma. Hematol Oncol Clin North Am *29*: 641, 2015.

89 Makohon-Moore A and Iacobuzio-Donahue CA: Pancreatic cancer biology and genetics from an evolutionary perspective. Nat Rev Cancer *16*: 553-565, 2016.

90 Vogelstein B and Kinzler KW: The path to cancer --three strikes and you're out. N Engl J Med *373*: 1895-1898, 2015.

91 Stolzenberg-Solomon RZ and Amundadottir LT: Epidemiology and inherited predisposition for sporadic pancreatic adenocarcinoma. Hematol Oncol Clin North Am *29*: 619-640, 2015.

92 Gukovsky I, Li N, Todoric J, Gukovskaya A and Karin M: Inflammation, autophagy, and obesity: Common features in the pathogenesis of pancreatitis and pancreatic cancer. Gastroenterology *144*: 1199-1209, 2013.

93 Rahn S, Zimmermann V, Viol F, Knaack H, Stemmer K, Peters L, Lenk L, Ungefroren H, Saur D, Schafer H, Helm O and Sebens S: Diabetes as risk factor for pancreatic cancer: Hyperglycemia promotes epithelial-mesenchymal-transition and stem cell properties in pancreatic ductal epithelial cells. Cancer Lett *415*: 129-150, 2018.

94 Kanda M, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B and Goggins M: Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology *142*: 730-733, 2012.

95 Philip B, Roland CL, Daniluk J, Liu Y, Chatterjee D, Gomez SB, Ji BA, Huang HJ, Wang HM, Fleming JB, Logsdon CD and Cruz-Monserrate Z: A high-fat diet activates oncogenic kras and cox2 to induce development of pancreatic ductal adenocarcinoma in mice. Gastroenterology *145*: 1449-1458, 2013.

96 Rubinson DA and Wolpin BM: Therapeutic approaches for metastatic pancreatic adenocarcinoma. Hemat Oncol Clin North Am *29*: 761-766, 2015.

97 Johansson B, Bardi G, Heim S, Mandahl N, Mertens F, Bak-Jensen E, Andren-Sandberg A and Mitelman F: Nonrandom chromosomal rearrangements in pancreatic carcinomas. Cancer *69*: 1674-1681, 1992.

98 Adsay NV, Dergham ST, Koppitch FC, Dugan MC, Mohamed AN, Vaitkevicius VK and Sarkar FH: Utility of fluorescence *in situ* hybridization in pancreatic ductal adenocarcinoma. Pancreas *18*: 111-116, 1999.

99 Chung DC, Smith AP, Louis DN, Graeme-Cook F, Warshaw AL and Arnold A: A novel pancreatic endocrine tumor suppressor gene locus on chromosome 3p with clinical prognostic implications. J Clin Invest *100*: 404-410, 1997.

100 Eskelinen M, Lipponen P, Collan Y, Marin S, Alhava E and Nordling S: Relationship between DNA ploidy and survival in patients with exocrine pancreatic cancer. Pancreas *6*: 90-95, 1991.

101 Stigliano V, Sanchez-Mete L, Martayan A, Diodoro M, Casini B, Sperduti I and Anti M: Early-onset colorectal cancer patients without family history are "at very low risk" for lynch syndrome. J Exp Clin Cancer Res *33*: 1, 2014.

102 Nagler C, Zanker KS and Dittmar T: Cell fusion, drug resistance and recurrence CSCs. Adv Exp Med Biol *714*: 173-182, 2011.

103 Searles SC, Santosa EK and Bui JD: Cell-cell fusion as a mechanism of DNA exchange in cancer. Oncotarget *9*: 6156-6173, 2018.

104 Carloni V, Mazzocca A, Mello T, Galli A and Capaccioli S: Cell fusion promotes chemoresistance in metastatic colon carcinoma. Oncogene *32*: 2649-2660, 2013.

105 Gonzalez-Gonzalez M, Garcia J, Alcazar JA, Gutierrez ML, Gonzalez LM, Bengoechea O, Abad MM, Santos-Briz A, Blanco O, Martin M, Rodriguez A, Fuentes M, Munoz-Bellvis L, Orfao A and Sayagues JM: Association between the cytogenetic profile of tumor cells and response to preoperative radiochemotherapy in locally advanced rectal cancer. Medicine *93*: e153, 2014.

106 Natrajan RC: Breast cancer heterogeneity: Parallel evolution or conscious uncoupling? J Pathol *237*: 1-3, 2015.

107 Toy W, Shen Y, Won H, Green B, Sakr RA, Will M, Li ZQ, Gala K, Fanning S, King TA, Hudis C, Chen D, Taran T, Hortobagyi G, Greene G, Berger M, Baselga J and Chandarlapaty S: ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet *45*: 1439-1445, 2013.

108 Goh JY, Feng M, Wang W, Oguz G, Yatim S, Lee PL, Bao Y, Lim TH, Wang P, Tam WL, Kodahl AR, Lyng MB, Sarma S, Lin SY, Lezhava A, Yap YS, Lim AST, Hoon DSB, Ditzel HJ, Lee SC, Tan EY and Yu Q: Chromosome 1q21.3 amplification is a trackable biomarker and actionable target for breast cancer recurrence. Nat Med *23*: 1319-1330, 2017.

109 Casuscelli J, Weinhold N, Gundem G, Wang L, Zabor EC, Drill E, Wang PI, Nanjangud GJ, Redzematovic A, Nargund AM, Manley BJ, Arcila ME, Donin NM, Cheville JC, Thompson RH, Pantuck AJ, Russo P, Cheng EH, Lee W, Tickoo SK, Ostrovnaya I, Creighton CJ, Papaemmanuil E, Seshan VE, Hakimi AA and Hsieh JJ: Genomic landscape and evolution of metastatic chromophobe renal cell carcinoma. JCI Insight *2*: 92688, 2017.

110 Sanfrancesco JM and Cheng L: Complexity of the genomic landscape of renal cell carcinoma: Implications for targeted therapy and precision immuno-oncology. Crit Rev Oncol Hematol *119*: 23-28, 2017.

111 Cantwell-Dorris ER, O'Leary JJ and Sheils OM: *BRAF*(V600E): Implications for carcinogenesis and molecular therapy. Mol Cancer Ther *10*: 385-394, 2011.

112 Dhomen N and Marais R: New insight into *BRAF* mutations in cancer. Curr Opin Genet Dev *17*: 31-39, 2007.

113 Yancovitz M, Litterman A, Yoon J, Ng E, Shapiro RL, Berman RS, Pavlick AC, Darvishian F, Christos P, Mazumdar M, Osman I and Polsky D: Intra- and inter-tumor heterogeneity of BRAF(V600E) mutations in primary and metastatic melanoma. Plos One *7*: 29336, 2012.

114 Lemech C, Infante J and Arkenau HT: The potential for *BRAF* V600 inhibitors in advanced cutaneous melanoma: Rationale and latest evidence. Ther Adv Med Oncol *4*: 61-73, 2012.

115 Spagnolo F, Ghiorzo P, Orgiano L, Pastorino L, Picasso V, Tornari E, Ottaviano V and Queirolo P: *BRAF*-mutant melanoma: Treatment approaches, resistance mechanisms, and diagnostic strategies. Onco Targets Ther *8*: 157-168, 2015.

116 Fedorenko IV and Smalley KS: The complexity of microenvironment-mediated drug resistance. Genes Cancer *6*: 367-368, 2015.

117 Whipple CA and Brinckerhoff CE: *BRAF*(V600E) melanoma cells secrete factors that activate stromal fibroblasts and enhance tumourigenicity. Br J Cancer *111*: 1625-1633, 2014.

118 Kodet O, Dvorankova B, Bendlova B, Sykorova V, Krajsova I, Stork J, Kucera J, Szabo P, Strnad H, Kolar M, Vlcek C, Smetana K and Lacina L: Microenvironment-driven resistance to B-Raf inhibition in a melanoma patient is accompanied by broad changes of gene methylation and expression in distal fibroblasts. Int J Mol Med *41*: 2687-2703, 2018.

119 Saini N, Roberts SA, Klimczak LJ, Chan K, Grimm SA, Dai SS, Fargo DC, Boyer JC, Kaufmann WK, Taylor JA, Lee E, Cortes-Ciriano I, Park PJ, Schurman SH, Malc EP, Mieczkowski PA and Gordenin DA: The impact of environmental and endogenous damage on somatic mutation load in human skin fibroblasts. Plos Genetics *12*: 1006385, 2016.

120 Blokx WA, van Dijk MC and Ruiter DJ: Molecular cytogenetics of cutaneous melanocytic lesions – diagnostic, prognostic and therapeutic aspects. Histopathology *56*: 121-132, 2010.

121 McGranahan N and Swanton C: Clonal heterogeneity and tumor evolution: Past, present, and the future. Cell *168*: 613-628, 2017.

122 Anderson K, Lutz C, van Delft FW, Bateman CM, Guo YP, Colman SM, Kempski H, Moorman AV, Titley I, Swansbury J, Kearney L, Enver T and Greaves M: Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature *469*: 356-361, 2011.

123 Mankuzhy NP, Walling E, Anderson B and Mody R: Cryptic etv6-abl1 fusion and mll2 truncation revealed by integrative clinical sequencing in multiply relapsed acute lymphoblastic leukemia. J Pediatr Hematol Oncol, doi: 10.1097/MPH.0000000000001249, 2018.

124 Martin-Lorenzo A, Auer F, Chan LN, Garcia-Ramirez I, Gonzalez-Herrero I, Rodriguez-Hernandez G, Bartenhagen C, Dugas M, Gombert M, Ginzel S, Blanco O, Orfao A, Alonso-Lopez D, Rivas JL, Garcia-Cenador MB, Garcia-Criado FJ, Muschen M, Sanchez-Garcia I, Borkhardt A, Vicente-Duenas C and Hauer J: Loss of PAX5 exploits SCA1-BCR-ABL(p190) susceptibility to confer the metabolic shift essential for PB-ALL. Cancer Res *78*: 2669-2679, 2018.

125 Walsh KM, de Smith AJ, Hansen HM, Smirnov IV, Gonseth S, Endicott AA, Xiao JQ, Rice T, Fu CH, McCoy LS, Lachance DH, Eckel-Passow JE, Wiencke JK, Jenkins RB, Wrensch MR, Ma XM, Metayer C and Wiemels JL: A heritable missense polymorphism in *CDKN2A* confers strong risk of childhood acute lymphoblastic leukemia and is preferentially selected during clonal evolution. Cancer Res *75*: 4884-4894, 2015.

126 Bateman CM, Colman SM, Chaplin T, Young BD, Eden TO, Bhakta M, Gratias EJ, van Wering ER, Cazzaniga G, Harrison CJ, Hain R, Ancliff P, Ford AM, Kearney L and Greaves M: Acquisition of genome-wide copy number alterations in monozygotic twins with acute lymphoblastic leukemia. Blood *115*: 3553-3558, 2010.

127 Soverini S, Hochhaus A, Nicolini FE, Gruber F, Lange T, Saglio G, Pane F, Muller MC, Ernst T, Rosti G, Porkka K, Baccarani M, Cross NCP and Martinelli G: BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: Recommendations from an expert panel on behalf of European Leukemianet. Blood *118*: 1208-1215, 2011.

128 Chopade P and Akard LP: Improving outcomes in chronic myeloid leukemia over time in the era of tyrosine kinase inhibitors. Clin Lymphoma Myeloma Leuk *18*: 710-723, 2018.

129 Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Lowenberg B and Bloomfield CD: Diagnosis and management of aml in adults: 2017 ELN recommendations from an international expert panel. Blood *129*: 424-447, 2017.

130 Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson AG, Hoadley K, Triche TJ, Laird PW, Baty JD, Fulton LL, Fulton R, Heath SE, Kalicki-Veizer J, Kandoth C, Klco JM, Koboldt DC, Kanchi KL, Kulkarni S, Lamprecht TL, Larson DE, Lin L, Lu C, McLellan MD, McMichael JF, Payton J, Schmidt H, Spencer DH, Tomasson MH, Wallis JW, Wartman LD, Watson MA, Welch J, Wendl MC, Ally A, Balasundaram M, Birol I, Butterfield Y, Chiu R, Chu A, Chuah E, Chun HJ, Corbett R, Dhalla N, Guin R, He A, Hirst C, Hirst M, Holt RA, Jones S, Karsan A, Lee D, Li HI, Marra MA, Mayo M, Moore RA, Mungall K, Parker J, Pleasance E, Plettner P, Schein J, Stoll D, Swanson L, Tam A, Thiessen N, Varhol R, Wye N, Zhao YJ, Gabriel S, Getz G, Sougnez C, Zou LH, Leiserson MDM, Vandin F, Wu HT, Applebaum F, Baylin SB, Akbani R, Broom BM, Chen K, Motter TC, Nguyen K, Weinstein JN, Zhang NZ, Ferguson ML, Adams C, Black A, Bowen J, Gastier-Foster J, Grossman T, Lichten-Berg T, Wise L, Davidsen T, Demchok JA, Shaw KRM, Sheth M, Sofia HJ, Yang LM, Downing JR, Eley G, Alonso S, Ayala B, Baboud J, Backus M, Barletta SP, Berton DL, Chu AL, Girshik S, Jensen MA, Kahn A, Kothiyal P, Nicholls MC, Pihl TD, Pot DA, Raman R, Sanbhadti RN, Snyder EE, Srinivasan D, Walton JS, Wan YH, Wang ZN, Issa JPJ, Le Beau M, Carroll M, Kantarjian H, Kornblau S, Bootwalla MS, Lai PH, Shen H, Van den Berg DJ, Weisenberger DJ, Link DC, Walter MJ, Ozenberger BA, Mardis ER, Westervelt P, Graubert TA, DiPersio JF, Wilson RK and Network CGAR: Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. N Engl J Med *368*: 2059-2074, 2013.

131 Churpek JE, Pyrtel K, Kanchi KL, Shao J, Koboldt D, Miller CA, Shen D, Fulton R, O'Laughlin M, Fronick C, Pusic I, Uy GL, Braunstein EM, Levis M, Ross J, Elliott K, Heath S, Jiang

A, Westervelt P, DiPersio JF, Link DC, Walter MJ, Welch J, Wilson R, Ley TJ, Godley LA and Graubert TA: Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. Blood *126*: 2484-2490, 2015.

132 Jan M, Snyder TM, Corces-Zimmerman MR, Vyas P, Weissman IL, Quake SR and Majeti R: Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. Sci Transl Med *4*: 149ra118, 2012.

133 Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, McLeod JL, Doedens M, Medeiros JJ, Marke R, Kim HJ, Lee K, McPherson JD, Hudson TJ, Consortium HP-LGP, Brown AM, Yousif F, Trinh QM, Stein LD, Minden MD, Wang JC and Dick JE: Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature *506*: 328-333, 2014.

134 Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC and Majeti R: Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. Proc Natl Acad Sci USA *111*: 2548-2553, 2014.

135 Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z, Mollica L, Li J, Viale A, Heguy A, Hassimi M, Socci N, Bhatt PK, Gonen M, Mason CE, Melnick A, Godley LA, Brennan CW, Abdel-Wahab O and Levine RL: Recurrent somatic *TET2* mutations in normal elderly individuals with clonal hematopoiesis. Nat Genet *44*: 1179-1181, 2012.

136 Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, McMichael JF, Schmidt HK, Yellapantula V, Miller CA, Ozenberger BA, Welch JS, Link DC, Walter MJ, Mardis ER, Dipersio JF, Chen F, Wilson RK, Ley TJ and Ding L: Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med *20*: 1472-1478, 2014.

137 Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M and Vardiman JW: The 2016 revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. Blood *127*: 2391-2405, 2016.

138 Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, Gundem G, Van Loo P, Martincorena I, Ganly P, Mudie L, McLaren S, O'Meara S, Raine K, Jones DR, Teague JW, Butler AP, Greaves MF, Ganser A, Dohner K, Schlenk RF, Dohner H and Campbell PJ: Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med *374*: 2209-2221, 2016.

139 Oracki SA, Walker JA, Hibbs ML, Corcoran LM and Tarlinton DM: Plasma cell development and survival. Immunological Reviews *237*: 140-159, 2010.

140 Seifert M, Scholtysik R and Kuppers R: Origin and pathogenesis of B-cell lymphomas. Methods Mol Biol *971*: 1-25, 2013.

141 Morgan GJ, Walker BA and Davies FE: The genetic architecture of multiple myeloma. Nat Rev Cancer *12*: 335-348, 2012.

142 Bianchi G and Munshi NC: Pathogenesis beyond the cancer clone(s) in multiple myeloma. Blood 125: 3049-3058, 2015.

143 Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, Harview CL, Brunet JP, Ahmann GJ, Adli M, Anderson KC, Ardlie KG, Auclair D, Baker A, Bergsagel PL, Bernstein BE, Drier Y, Fonseca R, Gabriel SB, Hofmeister CC, Jagannath S, Jakubowiak AJ, Krishnan A, Levy J, Liefeld T, Lonial S, Mahan S, Mfuko B, Monti S, Perkins LM, Onofrio R, Pugh TJ, Rajkumar SV, Ramos AH, Siegel DS, Sivachenko A, Stewart AK, Trudel S, Vij R, Voet D, Winckler W, Zimmerman T, Carpten J, Trent J, Hahn WC, Garraway LA, Meyerson M, Lander ES, Getz G and Golub TR: Initial genome sequencing and analysis of multiple myeloma. Nature *471*: 467-472, 2011.

144 Egan JB, Shi CX, Tembe W, Christoforides A, Kurdoglu A, Sinari S, Middha S, Asmann Y, Schmidt J, Braggio E, Keats JJ, Fonseca R, Bergsagel PL, Craig DW, Carpten JD and Stewart AK: Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides. Blood *120*: 1060-1066, 2012.

145 Walker BA, Wardell CP, Melchor L, Brioli A, Johnson DC, Kaiser MF, Mirabella F, Lopez-Corral L, Humphray S, Murray L, Ross M, Bentley D, Gutierrez NC, Garcia-Sanz R, Miguel JS, Davies FE, Gonzalez D and Morgan GJ: Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms. Leukemia *28*: 384-390, 2014.

146 Walker BA, Wardell CP, Melchor L, Hulkki S, Potter NE, Johnson DC, Fenwick K, Kozarewa I, Gonzalez D, Lord CJ, Ashworth A, Davies FE and Morgan GJ: Intraclonal heterogeneity and distinct molecular mechanisms characterize the development of t(4;14) and t(11;14) myeloma. Blood *120*: 1077-1086, 2012.

147 Keats JJ, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, Van Wier S, Blackburn PR, Baker AS, Dispenzieri A, Kumar S, Rajkumar SV, Carpten JD, Barrett M, Fonseca R, Stewart AK and Bergsagel PL: Clonal competition with alternating dominance in multiple myeloma. Blood *120*: 1067-1076, 2012.

148 Magrangeas F, Avet-Loiseau H, Gouraud W, Lode L, Decaux O, Godmer P, Garderet L, Voillat L, Facon T, Stoppa AM, Marit G, Hulin C, Casassus P, Tiab M, Voog E, Randriamalala E, Anderson KC, Moreau P, Munshi NC and Minvielle S: Minor clone provides a reservoir for relapse in multiple myeloma. Leukemia *27*: 473-481, 2013.

149 Godwin JW and Rosenthal N: Scar-free wound healing and regeneration in amphibians: Immunological influences on regenerative success. Differentiation *87*: 66-75, 2014.

150 Xue ML and Jackson CJ: Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv Wound Care *4*: 119-136, 2015.

151 Kishi K, Okabe K, Shimizu R and Kubota Y: Fetal skin possesses the ability to regenerate completely: Complete regeneration of skin. Keio J Med *61*: 101-108, 2012.

152 Ogawa R: Keloid and hypertrophic scars are the result of chronic inflammation in the reticular *dermis*. Int J Mol Sci *18*: 606, 2017.

153 Jumper N, Paus R and Bayat A: Functional histopathology of keloid disease. Histol Histopathol *30*: 1033-1057, 2015.

154 Rees PA, Greaves NS, Baguneid M and Bayat A: Chemokines in wound healing and as potential therapeutic targets for reducing cutaneous scarring. Adv Wound Care *4*: 687-703, 2015.

155 van den Broek LJ, Limandjaja GC, Niessen FB and Gibbs S: Human hypertrophic and keloid scar models: Principles, limitations and future challenges from a tissue engineering perspective. Expl Dermatol *23*: 382-386, 2014.

156 Leavitt T, Hu MS, Marshall CD, Barnes LA, Lorenz HP and Longaker MT: Scarless wound healing: Finding the right cells and signals. Cell Tissue Res *365*: 483-493, 2016.

157 Ehrlich HP, Desmouliere A, Diegelmann RF, Cohen IK, Compton CC, Garner WL, Kapanci Y and Gabbiani G: Morphological and immunochemical differences between keloid and hypertrophic scar. Am J Pathol *145*: 105-113, 1994.

158 Lee JY, Yang CC, Chao SC and Wong TW: Histopathological differential diagnosis of keloid and hypertrophic scar. Am J Dermatopathol *26*: 379-384, 2004.

159 Dvorak HF, Flier J and Frank H: Tumors - wounds that do not heal - similarities between tumor stroma generation and wound-healing. N Engl J Med *315*: 1650-1659, 1986.

160 Dvorankova B, Szabo P, Lacina L, Gal P, Uhrova J, Zima T, Kaltner H, Andre S, Gabius HJ, Sykova E and Smetana K: Human galectins induce conversion of dermal fibroblasts into myofibroblasts and production of extracellular matrix: Potential application in tissue engineering and wound repair. Cells Tissues Organs *194*: 469-480, 2011.

161 Smetana K, Szabo P, Gal P, Andre S, Gabius HJ, Kodet O and Dvorankova B: Emerging role of tissue lectins as micro-environmental effectors in tumors and wounds. Histol and Histopathol *30*: 293-309, 2015.

162 Valach J, Fik Z, Strnad H, Chovanec M, Plzak J, Cada Z, Szabo P, Sachova J, Hroudova M, Urbanova M, Steffl M, Paces J, Mazanek J, Vlcek C, Betka J, Kaltner H, Andre S, Gabius HJ, Kodet R, Smetana K, Gal P and Kolar M: Smooth muscle actin-expressing stromal fibroblasts in head and neck squamous cell carcinoma: Increased expression of galectin-1 and induction of poor prognosis factors. Int J Cancer *131*: 2499-2508, 2012.

163 Kojima Y, Acar A, Eaton EN, Mellody KT, Scheel C, Ben-Porath I, Onder TT, Wang ZC, Richardson AL, Weinberg RA and Orimo A: Autocrine tgf-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. Proc Natl Acad Sci USA *107*: 20009-20014, 2010.

164 Pierce GF, Vande Berg J, Rudolph R, Tarpley J and Mustoe TA: Platelet-derived growth factor-bb and transforming growth factor beta 1 selectively modulate glycosaminoglycans, collagen, and myofibroblasts in excisional wounds. Am J Pathol *138*: 629-646, 1991.

165 Ren Y, Zhou X, Wei Z, Lin W, Fan B and Feng S: Efficacy and safety of triamcinolone acetonide alone and in combination with 5-fluorouracil for treating hypertrophic scars and keloids: A systematic review and meta-analysis. Int Wound J *14*: 480-487, 2017.

166 Su L, Fu L, Li Y, Yang F, Zhang M and Hu D: Disruption of the association between drug transporter and actin cytoskeleton abolishes drug resistance in hypertrophic scar. Oncotarget *8*: 2617-2627, 2017.

167 Jarkovska K, Dvorankova B, Halada P, Kodet O, Szabo P, Gadher SJ, Motlik J, Kovarova H and Smetana K: Revelation of fibroblast protein commonalities and differences and their possible roles in wound healing and tumourigenesis using co-culture models of cells. Biol Cell *106*: 203-218, 2014.

168 Deonarine K, Panelli MC, Stashower ME, Jin P, Smith K, Slade HB, Norwood C, Wang E, Marincola FM and Stroncek DF: Gene expression profiling of cutaneous wound healing. J Transl Med *5*: 11, 2007.

169 Werner S, Krieg T and Smola H: Keratinocyte-fibroblast interactions in wound healing. J Invest Dermatol *127*: 998-1008, 2007.

170 Klima J, Lacina L, Dvorankova B, Herrmann D, Carnwath JW, Niemann H, Kaltner H, Andre S, Motlik J, Gabius HJ and Smetana K Jr.: Differential regulation of galectin expression/reactivity during wound healing in porcine skin and in cultures of epidermal cells with functional impact on migration. Physiol Res *58*: 873-884, 2009.

171 Perzelova V, Varinska L, Dvorankova B, Szabo P, Spurny P, Valach J, Mojzis J, Andre S, Gabius HJ, Smetana K Jr. and Gal P: Extracellular matrix of galectin-1-exposed dermal and tumor-associated fibroblasts favors growth of human umbilical vein endothelial cells *in vitro*: A short report. Anticancer Res *34*: 3991-3996, 2014.

172 Bull JJ and Barrick JE: Arresting evolution. Trends Genet *33*: 910-920, 2017.

173 Purushotham AD and Sullivan R: Darwin, medicine and cancer. Ann Oncol *21*: 199-203, 2010.

174 Schwartz R and Schaffer AA: The evolution of tumour phylogenetics: Principles and practice. Nat Rev Genet *18*: 213-229, 2017.

175 Beerenwinkel N, Schwarz RF, Gerstung M and Markowetz F: Cancer evolution: Mathematical models and computational inference. Syst Biol *64*: E1-E25, 2015.

176 Wang JG, Cazzato E, Ladewig E, Frattini V, Rosenbloom DIS, Zairis S, Abate F, Liu ZQ, Elliott O, Shin YJ, Lee JK, Lee IH, Park WY, Eoli M, Blumberg AJ, Lasorella A, Nam DH, Finocchiaro G, Iavarone A and Rabadan R: Clonal evolution of glioblastoma under therapy. Nat Genet *48*: 768-776, 2016.

177 Bakhoum SF and Landau DA: Chromosomal instability as a driver of tumor heterogeneity and evolution. Cold Spring Harb Perspect Med *7*: a029611, 2017.

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