Mutation Research-Reviews in Mutation Research 777 (2018) 1–18

Contents lists available at ScienceDirect

Mutation Research-Reviews in Mutation Research

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

Review

Multiclonal tumor origin: Evidence and implications

Barbara L. Parsons

US Food and Drug Administration, National Center for Toxicological Research, Division of Genetic and Molecular Toxicology, 3900 NCTR Rd., Jefferson, AR 72079, United States

ARTICLE INFO

Keywords:
Multiclonal tumor origin
Polyclonal
Clonality
Tumor heterogeneity
Tumor initiation
X chromosome inactivation

ABSTRACT

An accurate understanding of the clonal origins of tumors is critical for designing effective strategies to treat or prevent cancer and for guiding the field of cancer risk assessment. The intent of this review is to summarize evidence of multiclonal tumor origin and, thereby, contest the commonly held assumption of monoclonal tumor origin. This review describes relevant studies of X chromosome inactivation, analyses of tumor heterogeneity using other markers, single cell sequencing, and lineage tracing studies in aggregation chimeras and engineered rodent models. Methods for investigating tumor clonality have an inherent bias against detecting multiclonality. Despite this, multiclonality has been observed within all tumor stages and within 53 different types of tumors. For myeloid tumors, monoclonal tumor origin may be the predominant path to cancer and a monoclonal tumor origin cannot be ruled out for a fraction of other cancer types. Nevertheless, a large body of evidence supports the conclusion that most cancers are multiclonal in origin. Cooperation between different cell types and between clones of cells carrying different genetic and/or epigenetic lesions is discussed, along with how polyclonal tumor origin can be integrated with current perspectives on the genesis of tumors. In order to develop biologically sound and useful approaches to cancer risk assessment and precision medicine, mathematical models of carcinogenesis are needed, which incorporate multiclonal tumor origin and the contributions of spontaneous mutations in conjunction with the selective advantages conferred by particular mutations and combinations of mutations. Adherence to the idea that a growth must develop from a single progenitor cell to be considered neoplastic has outlived its usefulness. Moving forward, explicit examination of tumor clonality, using advanced tools, like lineage tracing models, will provide a strong foundation for future advances in clinical oncology and better training for the next generation of oncologists and pathologists.

1. Introduction

Evidence supporting multiclonal tumor origin was reviewed in 2008 in a publication entitled, “Many different tumor types have polyclonal tumor origin: Evidence and implications” [1]. A timeline of the critical studies discussed in that review are presented in Fig. 1 and led to the conclusion that tumor initiation often involves the interaction between two or more distinct clones of cells. A massive expansion of research on tumor heterogeneity has occurred in the eight years following the publication of this article, driven in large part by the impact of tumor heterogeneity on personalized cancer therapy. Despite the clonal origin of tumors being a critical aspect of the theory underpinning cancer research, only a small fraction of oncology publications address the concept of multiclonal tumor origin in a direct manner. In order to draw attention to the discrepancy between continued acceptance of monoclonal tumor origin and available data, the following areas are reviewed here: 1) the current state of scientific acceptance of monoclonal versus multiclonal tumor origin, 2) X chromosome inactivation (XCI) studies published since 2008, 3) evidence regarding the clonal origins of tumors obtained using other markers (studies published in 2010 or later), 4) data obtained using single cell sequencing, 5) data obtained using aggregation chimeras and lineage tracing engineered rodent models, and 6) evidence of clonal interactions driving tumor initiation and progression. Finally, the compatibility of multiclonal tumor origin with various aspects of tumorigenesis is considered and the implications of multiclonal tumor origin for chemical safety assessment and for treating and preventing cancer are discussed.

2. Current state of acceptance/understanding regarding the clonal origins of tumors

According to educational materials provided by the National Institutes of Health [2], monoclonal tumor origin means that tumors are derived from a single ancestral cell that underwent conversion from a normal to a cancerous state. Biclonal, oligoclonal, multiclonal, and polyclonal tumor origin are terms that denote tumors developed from

E-mail address: Barbara.parsons@fda.hhs.gov.

https://doi.org/10.1016/j.mrrev.2018.05.001
Received 27 February 2018; Received in revised form 11 April 2018; Accepted 5 May 2018
Available online 06 May 2018

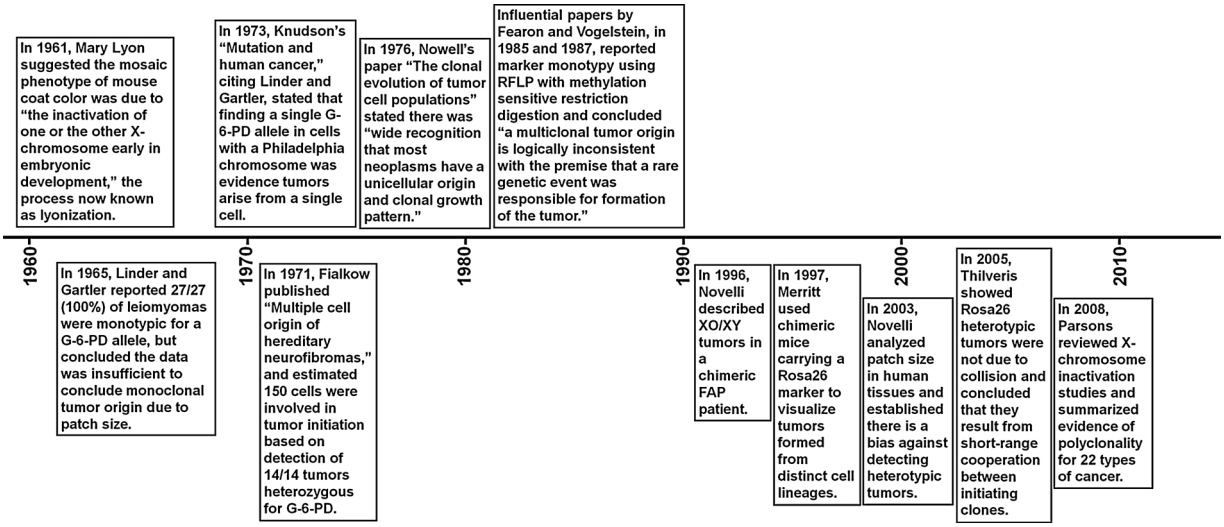

Fig. 1. Impactful studies using XCI to investigate the clonal origins of tumors. Studies interpreted as providing support for monoclonal tumor origin are presented above the timeline [180–183] (except [184] which drew no conclusions relevant to cancer). Studies interpreted as providing support for multiclonal tumor origin are presented below the timeline [1,29–32,185,186].

two, a few, multiple, or many progenitor cells, respectively. In this review, the term "multiclonal tumor origin" will be used to convey tumor development from an indeterminate number of distinct clones of cells greater than one. An accurate understanding of the clonal origins of cancer is needed to efficiently advance personalized cancer treatment and, thereby, reduce cancer deaths. Treatment of cancers with monotherapies may be justified if cancers are monoclonal in origin and the progenitor clone carries a genetic lesion in a pathway that can be targeted with a drug. However, treating patients with a molecularly targeted monotherapy is unlikely to be effective if patients' tumors developed via multiclonal initiation, because minor untargeted clones can drive therapeutic resistance [3,4].

Monoclonal tumor origin was espoused definitively in the cancer literature for many years. The 2008 review [1] described how the perceived rarity of somatic mutations contributed to the conclusion that tumors must be monoclonal in origin (see Fig. 1), and how a cancer mutator phenotype was invoked to explain the large number of mutations observed in putatively monoclonal tumors. The review documented cancer texts published between 2001 and 2006, as well as teaching materials available on the National Cancer Institute (NCI) website in 2008, which promulgated the concept that cancers arise from a single cell. More recent reference materials, in aggregate, include fewer direct assertions of monoclonal tumor origin, while at the same time they rarely address the possibility of multiclonal tumor origin.

To document the current state of scientific acceptance of monoclonal versus multiclonal tumor origin, a survey of oncology and medical textbooks published between 2011 and 2017 was conducted. Six cancer textbooks included a discussion of the molecular genetics of cancer initiation and, together, capture the current state of ambiguity surrounding the topic. *Cancer: Principles & Practice of Oncology: Primer of the Molecular Biology of Cancer* [5] refers to the work of Rudolph Virchow and states "nearly 150 years later, it is now well accepted that cancer is a genetic disease that arises from the clonal expansion of a single neoplastic cell." The subsequent text presents two theories to explain how the descendants of the initiating cell become heterogeneous with respect to their proliferative abilities: 1) a stochastic theory (phenotypic variation produced in biologically-equivalent cells by extrinsic and/or intrinsic factors) and 2) cancer stem cell (CSC) theory. *Principles of Molecular Diagnostics and Personalized Cancer Medicine* [6] describes a progression of scientific understanding from the multistep model of carcinogenesis proposed by Nowell [7] to the CSC concept. The CSC model provides a framework to explain phenotypic variability within individuals cancers [5,6]. The text does not address directly the clonal origins of tumors, although it does say "thus in cancers, as opposed to normal tissues, a stem cell produces many more malignant stem cells than they differentiate" [6].

The Molecular Basis of Cancer [8] describes the discoveries that led to the conclusion that cancer is a genetic disorder, with accumulation of mutations and Darwinian evolution occurring during progression, as per the model of multistep tumorigenesis. The text is silent on the issue of tumor clonality. Conversely, *The Biology of Cancer* [9] describes the science that led to the conclusion that "tumors are monoclonal growths," including discussion of X chromosome inactivation, glucose-6-phosphate dehydrogenase (G6PD), immunoglobulin rearrangement, and translocations as markers of cell lineage. The text recognizes that interpretation of such markers would be irrelevant if differences in clonal proliferation produced apparently homotypic cancers from multiclonal initiating events (pseudomonoclonality discussed in [10]). Nevertheless, Dr. Weinberg concludes there "is a widespread consensus that the vast majority of human tumors are monoclonal growths descended from single progenitor cells that took the first small steps to becoming cancerous."

The treatment of cancer biology and genetics found in *Goldman-Cecil Medicine* [11] presents the multistep model of tumorigenesis as involving random mutations, with tumor evolution as a consequence of natural selection/clonal selection of mutant cell populations. This text recognizes that "a tumor does not solely consist of transformed cancer cells but represents a complex tissue that also includes a wide array of stromal cells." The authors [11] explain that "cancer progression occurs within the context of complex interactions between multiple cell types," including inflammatory cells and immune suppressor cells and that "the stochastic nature of oncogenic mutations supports genetic drift and the coevolution of cancer cells with different genetic lesions within a tumor." Further, Black and Cowan [11] note that variations in microenvironment and abilities of neoplastic cells to recruit other cell types "produces different evolutionary pressures that select for regional heterogeneity" within a cancer. The four most frequently cited explanations for how a phenotypically heterogeneous tumor develops from the progeny of a single initiated cell (not mutually exclusive) are depicted in Fig. 2; these include sequential accumulation of mutations with subsequent clonal selection (Fig. 2A), cooperation between malignant and non-malignant cell types (Fig. 2B), epithelial to mesenchymal transition (Fig. 2C), and proliferation and differentiation of the progeny of a single mutant stem cell (Fig. 2D).

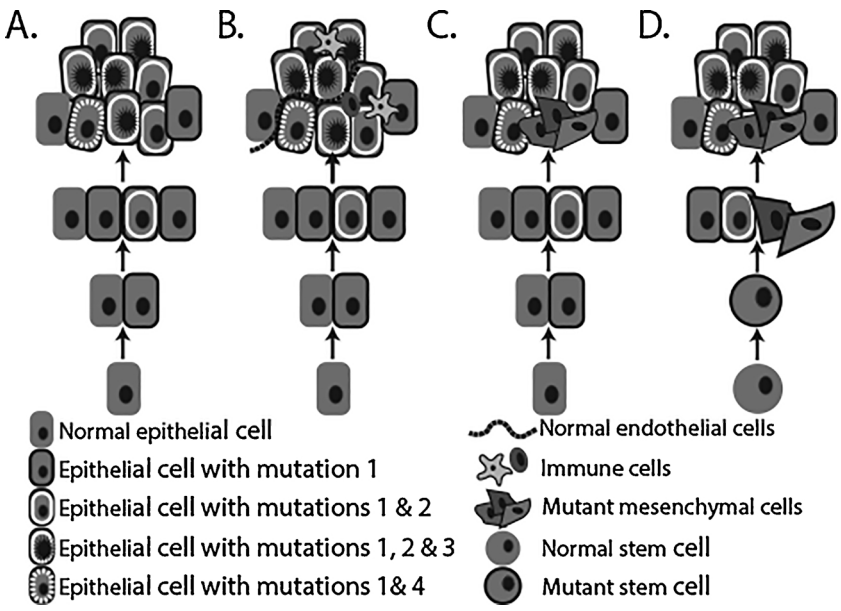

Fig. 2. Four common explanations for how monoclonal tumor origin results in phenotypically and genetically heterogeneous tumors. Depicted are the primary premise of monoclonal tumor origin, that all malignant cells in a tumor are the progeny of a single initiated cell, as well as early steps in tumor initiation and progresssion. It has been claimed that heterogeneity of monoclonal tumors arises through mutation accumulation and clonal selection (A), through cooperation between malignant and non-malignant cell types (B), through epithelial to mesenchymal or mesenchymal to epithelial transitions (C), and through proliferation and subsequent differentiation of a mutant stem cell (D).

When initially reviewed [1], the NCI website "Understanding Cancer – Teacher's Guide" provided: 1) a schematic comparing monoclonal and multiclonal tumor origin, 2) the statement "direct evidence supporting the monoclonal origin of virtually all malignant tumors has been difficult to acquire because most tumor cells lack obvious distinguishing marks that scientists can use to demonstrate their clonal relationship. There is, however, one cellular marker that scientists can use as an indication of such relationships: the inactivated X chromosome that occurs in almost all of the body cells of a human female," and 3) the conclusion that "virtually all malignant tumors are monoclonal in origin." This same information is contained in an educational package provided by NIH (revised in 2012), which includes a figure showing tumor development from a single cell and the text "cancer begins when a cell breaks free from the normal restraints on cell division and begins to follow its own agenda for proliferation. All of the cells produced by division of this first, ancestral cell and its progeny also display inappropriate proliferation" [2]. However, NCI's "Understanding Cancer" website (updated February 2015) includes a figure showing progression of a whole tissue toward cancer and states only that "Each person's cancer has a unique combination of genetic changes. As the cancer continues to grow, additional changes will occur. Even within the same tumor, different cells may have different genetic changes" [12].

This comparison of reference materials illustrates that over the past ten or fifteen years there has been a small shift away from dogmatic acceptance that existing evidence proves monoclonal tumor origin. But, even when monoclonal tumor origin is not specifically espoused, neither is the concept of polyclonal or multiclonal tumor origin presented. Furthermore, the scarcity of direct discussion of monoclonal versus multiclonal tumor origin in reference material (or as the subject of direct experimentation in the primary literature) suggests that the scientific community no longer considers this a critical research question. This may be shortsighted because understanding the earliest stages of tumor initiation has the potential to shape strategies for cancer prevention and treatment. Computational modeling of how tumor heterogeneity arises, for example, is an approach that can be used to understand the likelihood that resistance to treatment may occur. Mathematical models have been developed to deconvolute massively parallel sequencing data and deduce how somatic mutations accumulated in cell lineages that eventually give rise to a tumor. Such models initially incorporated the assumption of monoclonal tumor origin [13–15]. More recently, using data from multiple regions of a single tumor or from multiple single tumor cells, methods have been developed to describe tumor origins that include the possibilities of multiple cells of origin and tumor initiation being triggered by epigenetic traits [16].

3. X chromosome inactivation and assessment of tumor clonality

Analysis of X-chromosome inactivation was central to early interpretations of tumor clonality and led to monoclonality being broadly held as a property of neoplastic growths. Cheng et al. wrote, "As monoclonality has been accepted as a fundamental feature of human cancer, clonality analysis through X-chromosome inactivation assay has been widely used for assessment of tumors" [17]. Indeed, X chromosome inactivation (XCI) is the most extensively used approach for investigating tumor clonality and involves a determination of whether the same X chromosome has been inactivated within a majority of the cells in a particular tissue sample (referred to as non-random XCI). Non-random versus random XCI is used to classify cell populations as neoplastic or reactive, respectively [18]. The X-linked markers analyzed include protein isoforms, transcribed mRNAs, and the methylation status of specific gene targets [19]. Beyond distinguishing neoplastic from reactive growths, XCI has been used to investigate whether multiple tumors within an organ have a common progenitor. Landmark publications regarding the development, use, and interpretation of methods for XCI-based clonality assessments are given in Fig. 1.

The *human androgen receptor* (*HUMARA*) gene is a target used frequently in clonality assessment by methylation-specific PCR. The gene is useful for this purpose because it is located on the X chromosome and its promoter contains CpG islands encompassing methylation-specific restriction enzyme cleavage sites upstream from a highly polymorphic region [containing (CAG)n repeats]. DNA from normal tissue (or blood) and potentially neoplastic tissue from the same patient are digested with a restriction enzyme incapable of cleaving methylated DNA (e.g., *HpaII*) [20]. Concurrent analysis of undigested DNA is performed to confirm the patient has two distinguishable alleles. PCR amplification of the *HUMARA* target, employing digested and undigested DNA samples generates allele-specific products that can be discriminated by size using gel or capillary electrophoresis [20]. If XCI is random (as is typically observed in DNA from normal tissue), then two different-length methylated bands will be observed in digested, as well as undigested, DNA samples (heterotypy). If the DNA was isolated from the clonal progeny of a single cell, then XCI will be non-random and only one of the two alleles will be methylated (i.e., protected from digestion) and, subsequently, amplified and observed (monotypy).

XCI is an active area of epigenetics research and interpretation of future and past clonality assessments based upon XCI should take recent developments into account. Although it was previously understood that the one X chromosome was inactivated by methylation, it is now understood that the process involves a complex bipartite methylation-demethylation program that generates an active X chromosome-specific hypomethylation at gene promoters and hypermethylation within gene bodies [21,22]. Also, it has been reported that 15% of genes escape XCI and demonstrate bi-allelic expression [23]. Not all genes, therefore, will be good candidates for XCI clonality assessment [22,24]. While it was originally assumed that XCI was random and that 50% of cells would have one or the other X chromosome inactivated, several studies have found non-random or "skewed' XCI patterns in normal tissues, where more than 75% of cells may have the same inactive X chromosome [25]. This skewing is positively associated with age [19,26,27]. It has been suggested that skewing reflects age-associated deregulation of methylation [28].

4. Pitfalls in clonality assessment by XCI

There are at least three significant obstacles for interpreting monotypy at an X-linker marker as evidence of monoclonality. The first major concern is the confounding effect of "patch size" [1,29–33]. Consider the hierarchical development that occurs to produce a given tissue. For the most part, once XCI occurs in a cell, the progeny that develop from that cell carry the same inherited pattern of XCI. "Patch size" refers to an area of an organ or tissue that develops from a single progenitor and, therefore, has the same X chromosome inactivated across the entire area (patches are depicted in Fig. 3 as white and gray areas). Thus, the extent of cell division following "lyonization" determines clonal patch size, with the potential for further skewing with aging, as mentioned above. Large and small patch sizes are depicted in Fig. 3A and B, respectively. Only tumors arising from clones of cells along the boundaries between patches have the potential to develop into multiclonal tumors that can be detected by XCI. If two different cell clones within a patch cooperated to initiate a tumor (for example, one clone carries a somatic *KRAS* mutation and another clone carries a somatic *TP53* mutation as initiating events), the tumor that developed would be scored as monoclonal (see Fig. 3A). Because multiclonal tumors will only be detected when they develop from clones at the juncture between XCI-distinguishable patches (see Fig. 3B), there is a bias against detecting tumor multiclonality by XCI, and the bias is greater when the patch size is large. To give a specific example, Novelli et al. [29] reported the crypt pair phenotype index (crypts adjacent to another with a distinguishable phenotype) of the female colon is 8.2%. From this they estimate that "to exclude the possibility that all adenomas are polyclonal in origin, every crypt in at least 43 adenomas would need to be shown to be monophenotypic" and "to exclude the possibility that 10% of adenomas are polyclonal 430 adenomas would need to be examined." Thus, most XCI studies, do not have sufficient power to conclude monoclonal tumor origin.

A second impediment to interpreting X-linked monotypy as evidence of monoclonal tumor origin is the potential for selective outgrowth of a clone during tumor progression. Fig. 3C depicts multiclonal tumor initiation, with one clone having a selective growth advantage. It has been demonstrated using computer simulations that small differences in the growth properties of founder clones could result in apparent near-monoclonality (pseudomonoclonality) within a relatively short period of time [34]. This caveat makes it nearly impossible to conclude monoclonal tumor origin from monotypy of X-linked markers in fully-developed cancers.

The third concern, somewhat related to the second, is the sensitivity of clonality assessment. The assay is confounded by the presence of non-tumor cells within a tumor (immune cells, endothelial cells, etc.). Interpretation of X chromosome-linked marker studies, therefore, hinges upon quantification of the two alleles and selecting the degree of disparity in allele abundance that when exceeded will be accepted as evidence of monotypy [20,35]. A correction factor is used to account for skewing observed in the undigested DNA. Typically, allele ratios greater than 4:1 are interpreted as evidence of monotypy [20]. Thus, XCI studies are unable to detect heterotypy when the minor cell lineage represents less than 25% of the total cell number in the sample. It is concluded, therefore, that XCI is a relatively insensitive approach for clonality assessment that favors detection of monotypy.

XCI analyses involving methylation sensitive enzyme cleavage and PCR may also be confounded by technical issues; these include incomplete digestion, selective PCR amplification of different-sized products, analysis of samples collected by microdissection that may not be representative of the whole tumor, and skewing of inactivated alleles due to normal aging or disease [18,19,35,36]. Given these pitfalls,

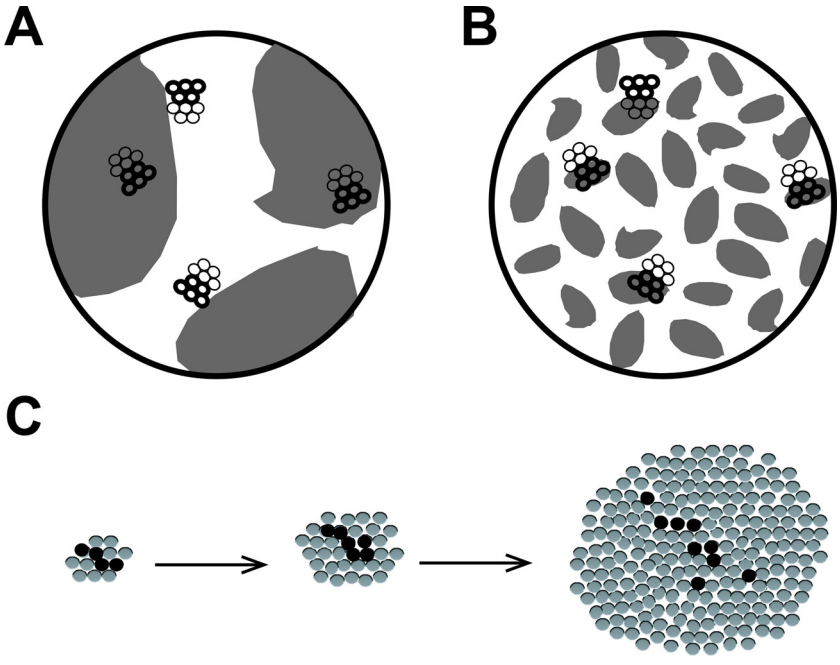

Fig. 3. Biases against detecting multiclonality by XCI are related to patch size and clonal selection during tumor development. Biclonal growths (cells in one clone have a thick border, cells in another clone have a thin border) are arrayed similarly in **A** and **B**, which have approximately equal proportions white and grey area (~50%) and represent patches of cells with one or the other X chromosome inactivated. In the case of large patch size (**A**) each of the biclonal growths will appear monotypic by XCI, whereas with small patch size (**B**) each of the biclonal growths will appear heterotypic by XCI. Preferential proliferation of one of two initiating clones is depicted in **C**, and illustrates how multiclonal tumor initiation can produce a pseudomonoclonal tumor mass.

approaches to improve clonality assessment based on XCI have been developed. Concurrent analysis of the methylation status of multiple gene targets and transcription based clonality assays are examples of approaches being pursued to improve clonality assessment [26,28,37–39]. Nevertheless, there is an ongoing debate in the scientific community as to the relative merits of the standard HUMARA assay and newer methodologies [18].

5. Interpretation and review of clonality assessments reported after 2007

The previous review [1] categorized XCI studies of preneoplastic lesions, adenomas and carcinomas (published in 2007 or earlier) as providing evidence of monoclonality or polyclonality. Given the biases in XCI analyses described above, monotypy of XCI-linked markers cannot be interpreted robustly as direct evidence of monoclonal tumor origin. Some detection of monotypy is expected for tumors that are multiclonal in origin and there is no scientific justification for concluding that samples containing a minor allele fraction of 0.24 or below are derived from a single cell. Such samples could as easily be multiclonal growths in which one clone has a selective advantage. In studies aimed at establishing monoclonal tumor origin, the scientific burden of proof should be to prove that 24% of the sample can be accounted for by contamination with “non-tumor cells,” otherwise samples should be categorized as heterotypic. The fact that this level of proof has not been required to conclude tumors are monoclonal in origin indicates there has been a confirmation bias in favor or reporting monotypy for samples investigators believe to be neoplastic. Importantly, detection of heterotypy in X-linked markers, even when the heterotypy is detected in a small proportion of the analyzed samples, provides direct evidence of multiclonal tumor origin.

A previous survey of XCI studies concluded that there was evidence of multiclonal tumor origin for at least 24 different types of human tumors, including tumors of bladder, blood, brain, breast, cervix, colon, endometrium, head and neck, lymph, nerve tissue, parathyroid gland, pituitary gland, prostate, skin, stomach, and thyroid gland [1]. To update this analysis, a literature review was conducted of XCI studies reported between 2007 and November 2017. The results of this literature search are presented in Tables 1 and 2. Table 1 identifies additional studies that detect heterotypy at an X-linked marker in at least some portion of the pre-neoplastic or tumor samples analyzed. Many of the studies summarized in Table 1 were conducted to ascertain whether spatially separated tumors within an organ or tissue are clonally-related or whether two morphologically-distinct portions of a single tumor have the same X chromosome inactivated. While such studies provide data on the XCI profiles of synchronous tumors, they may not discuss whether their results support monoclonal or multiclonal tumor origin [denoted as neutral (N) in Table 1]. Table 1 includes samples reported as heterotypic, as well as samples classified as monotypic but for which evidence of minor allele fraction(s) was provided.

Table 2 identifies additional studies that report monotypy at an X-linked marker in pre-neoplastic lesions or tumors. Previously collected data on clonality assessments [1] was combined with that provided in Tables 1 and 2. Combined, there are 15 publications with data indicating multiclonality exists in pre-neoplastic lesions of seven different tissues/organs (bone marrow, breast, cervix, colon, endometrium/uterus, liver and stomach). There are four publications reporting monotypic pre-neoplastic lesions. One of these was from lung [40]. Three studies in Table 2 report monotypic pre-neoplastic lesions [41–43] from tissues/organs (breast, liver, and uterus) for which evidence of multiclonal pre-neoplastic lesions have been reported [41–46] and, therefore, may reflect apparent monotypy arising after early lesion expansion. Alternatively, both modes of initiation may occur for some types of lesions.

Combining data from [1] with the updated data in Tables 1 and 2, there are 33 publications reporting evidence of multiclonality in 32 different benign tumors derived from 19 different tissues/organs, including adrenal gland, bladder, blood, brain, breast, colon, lung, kidney, oral cavity, ovary, pancreas, parathyroid, parotid gland, pituitary, spleen, skin, sympathetic nervous system, thyroid, and uterus. By contrast, there are 13 reports of monotypy for 14 different benign tumors. For three benign tumors (uterine leiomyoma, lung carcinoid and adenomatoid odontogenic tumor), evidence of multiclonality has also been reported. Considering all reports of benign tumors analyzed by XCI, thymus is the only tissue examined in which multiclonal tumors have not been detected.

Combining data from [1] with the updated data in Tables 1 and 2, there are 27 publications reporting evidence of multiclonality in 20 different malignancies derived from 12 different organs, including bladder, bone marrow, brain, breast, cervix, colon, liver, lung, ovary, skin, thyroid, and uterus. There are 20 reports of monotypy for 17 different malignant tumors. For nine of these lesions (adenocarcinoma of the cervix, bladder cancer, breast ductal carcinoma, chronic myeloid leukemia/myelodysplastic syndrome, endometrial carcinoma, hepatocellular carcinoma, Kaposi sarcoma, and lung adenocarcinoma), evidence of multiclonality has also been reported. Considering the tissues examined by XCI studies, there were only four tissues where malignant multiclonal tumors have not been detected (kidney, stomach, thymus and the urothelial tract).

As expected, this dataset includes examples of early stage tumors being characterized as heterotypic and later stage tumors being characterized as monotypic (e.g., myelodysplastic syndrome vs chromic myeloid leukemia). Combining the data in Tables 1 and 2 shows the percentage of multiclonal samples was decreased in tumors compared with pre-malignant samples [the percentages of multiclonal pre-neoplastic, benign, and malignant samples were 57.6% (163/283), 18.2% (110/602), and 24.4% (61/250), respectively]. In conclusion, even though XCI is a flawed approach for establishing that potentially oncogenic growths are monoclonal in origin, work using this approach has demonstrated that a wide range of pre-neoplastic, benign and malignant growths are derived from more than one cell lineage.

6. Clonality assessment using markers other than XCI

The breadth of markers (other than XCI) used to assess clonality in gastrointestinal cancers has been reviewed [47]. A review by Teixiera and Heim [48] summarized evidence of multiclonal tumors obtained from cytogenic analyses. Analyses of neutral passenger mutations, mitochondrial mutations, microsatellite markers, and epigenetic changes have been investigated as markers of clonality [24,49]. Studies conducted since 2007 that characterize the clonal composition of tumors through the analysis of markers other than XCI are summarized in Table 3. The approaches used include analysis of: karyotype, specific cancer driver mutations (CDMs), copy number variation, single nucleotide variants, cytogenetics, chromosome aberrations, microsatellite markers, and loss of heterozygosity. Of the 16 studies summarized in Table 3, five studies observed monoclonal tumor origin for: metaplastic/dysplastic lesions of the stomach, gastric adenocarcinomas, myelodysplastic syndrome, acute myeloid leukemia, and pleomorphic adenoma of the salivary gland. Eleven studies report multiclonal tumor origin for: Barrett’s esophageal lesions, B-cell precursor acute lymphocytic leukemia, breast carcinomas, colorectal microadenomas/adenomas/carcinomas, esophageal adenocarcinomas, hepatic carcinosarcomas, and prostate cancer.

Studies that use various genetic markers to address clonality also have potential biases against detecting polyclonality. There is the problem of pseudomonoclonality in a fully developed tumor of polyclonal origin and the possibility of convergent evolution causing monotypy in clones of separate lineage [50]. The possibility of convergent evolution should be considered, particularly for mutations/genetic events with strong phenotypic consequences and high prevalence (e.g., *KRAS*, *PIK3CA*, *VHL* mutations, and the Philadelphia chromosome) [50].

Table 1
XCI studies reporting evidence of multiclonality.[a]

Lesion or Tumor Type[b]	Method and X-linked Marker Examined[c]	# Heterotypic Samples/# Informative Samples Analyzed (percentage)	Author Conclusion[d]	Reference
Non-neoplastic lesions				
Erdheim-Chester disease	RFLP/methylation of *AR* and *PGK*	1/1 (100%)	Multi	[125]
Acquired aplastic anemia	RFLP/methylation of *AR*	6/7 (85.7%)	Multi & Mono	[126]
Pre-neoplastic lesions				
Bone marrow				
Myeloproliferative neoplasms	cDNA sequencing of X-linked SNPs from individually cultured colonies	2/6 (33.3%)	Multi	[127]
Breast				
Usual ductal hyperplasia	RFLP/methylation of *AR*	34/35 (97.1%)	Multi & Mono	[45]
Atypical ductal hyperplasia		26/39 (66.6%)	Multi & Mono	
Flat epithelial hyperplasia		23/26 (88.4%)	Multi & Mono	
Usual ductal hyperplasia	RFLP/methylation of *AR*	9/12 (75%)	Multi	[41]
Ductal intraepithelial hyperplasia 1A		5/18 (27.8%)		
Liver				
Focal nodular hyperplasia	RFLP/methylation of *AR*	9/13 (69.2%)	Mono	[44]
Focal nodular hyperplasia	RFLP/methylation of *AR*	9/9 (100%)	Multi	[46]
Low grade dysplastic nodules	RFLP/methylation of *AR*	38/57 (66.7%)	N	[42]
Uterus				
Cervical intraepithelial neoplasia w/ glandular dysplasia	RFLP/methylation of *AR*	4/4 (100.0%)	Multi & Mono	[43]
Lobular endocervical glandular hyperplasia (LEGH)	RFLP/methylation of *AR*	4/9 (44.4%)	Multi & Mono	[128]
Benign tumors				
Adrenal Gland				
Adrenal myelolipoma	RFLP/methylation of *AR*	4/4 (100%)	Multi	[129]
Blood				
Essential thrombocythemia	RFLP/methylation of *AR*	22/61 (36.1%)	Multi & Mono	[130]
Kidney				
Mixed epithelial and stromal tumors	RFLP/methylation of *AR*	7/19 (36.8%)	N	[131]
Lung				
Atypical carcinoid	RFLP/methylation of AR	1/2 (50.0%)	N	[132]
Mesothelial tumors				
Adenomatoid tumor	RFLP/methylation of *AR*	1/10 (10.0%)	Mono	[133]
Odontogenic tumors				
Mixoma	RFLP/methylation of *AR*	1/2 (50.0%)	Multi	[134]
Odontogenic keratocyst		2/5 (40.0%)		
Ovary				
Sertoli-Leydig cell tumor	RFLP/methylation of *AR*	7/10 (70.0%)	N	[135]
Pancreas				
Acinar cell cystadenoma	RFLP/methylation of *AR*	5/5 (100%)	Multi	[136]
Parotid gland				
Pleomorphic adenoma	RFLP/methylation of *AR*	1/1 (100%	Mono	[137]
Skin				
Seborrheic keratoses	RFLP/methylation of *AR*	1/8 (12.5%)	N	[103]
Spleen				
Hamartoma	RFLP/methylation of *AR*	2/2 (100%)	Multi & Mono	[138]
Cord capillary hemangioma		2/5 (40.0%)		
Myoid angioendothelioma		2/2 (100%)		
Sympathetic nervous system				
Neuroblastic tumors	RFLP/methylation of *AR* and *PGK*	14/17 (82.4%)	Multi	[139]
Uterus				
Leiomyoma	FACS cell sorting plus RFLP/methylation of *AR* or RNA-HUMARA	36/53 (67.9%) 2/25 (8.0%)	Mono	[140]
Malignant tumors				
Bladder				
Sarcomatoid urothelial carcinoma	RFLP/methylation of *AR*	3/8 (37.5)	Mono	[141]
Bone Marrow				
Myleodysplastic syndrome (low risk)	RFLP/methylation of *AR* and SNP pyrosequencing	2/25 (8.0%)	Mono	[26]
Brain				
Adamantinomatous craniopharyngioma	RFLP/methylation of *AR*	6/8 (75.0%)	Multi & Mono	[142]
Breast				
Invasive ductal carcinoma	RFLP/methylation of *AR*	1/1 (100%)	Multi	[143]
Ductal carcinoma in situ		14/37 (37.8%)	Mono	[45]
Cervix				
Minimal deviation adenocarcinoma w/ LEGH	RFLP/methylation of *AR*	2/4 (33.3%)	Multi & Mono	[128]
Neurodocrine carcinoma		7/15 (46.6%)	N	[135]
Liver				
Hepatocellular carcinoma	RFLP/methylation of *AR*	4/10 (40.0%)	N	[144]
Lung				

(*continued on next page*)

Table 1 (*continued*)

Lesion or Tumor Type[b]	Method and X-linked Marker Examined[c]	# Heterotypic Samples/# Informative Samples Analyzed (percentage)	Author Conclusion[d]	Reference
Malignant mesothelioma	RFLP/methylation of *AR*	13/13 (100%)	Multi	[145]
Adenocarcinoma		1/7 (14.3%)	Mono	[132]
Skin				
Kaposi sarcoma	RFLP/methylation of *AR* and PGK	1/12 (8.3%)	Mono	[146]
Melanoma	RFLP/methylation of *AR*	1/4 (25.0%)	N	[147]
Melanoma	RFLP/methylation of *AR*	3/11 (27.2%)	Multi	[148]
Thyroid				
Papillary thyroid carcinoma	RFLP/methylation of *AR*	3/6 (50.0%)	N	[149]

AR, androgen receptor gene; *PGK*, phosphoglycerate kinase gene.

[a] Studies involving known hereditary cancer syndromes and metastases are not included.

[b] Tumors without classification as benign or malignant were placed in the benign tumor section of the table.

[c] RFLP analyses rely upon digestion with methylation-sensitive restriction enzymes, with subsequent analysis of PCR product size or size of rtPCR product.

[d] Authors' conclusions are summarized as: Multi, multiclonal tumor; Mono, monoclonal tumor; Multi & Mono, both monoclonal and multiclonal tumors observed; and N, neutral.

7. Studies of tumor heterogeneity using analysis of single cells/nuclei

Interest in the analysis of single tumor cells has increased over recent years, driven, in large part, by the desire to identify truncal mutations in patient tumors that may be targeted by therapy [51]. The perceived strength of single cell analysis is that "intratumor heterogeneity provides a permanent record of the mutations that occurred during tumor growth, providing a window into time" [52]. Sequence analyses conducted on a bulk of admixed tumor cells may significantly degrade mutation detection sensitivity. According to Jeaniszewska et al. [53], "bulk tumor sequencing cannot accurately predict which mutations are present in the same or in different cells."

Studies that investigated intratumoral genetic heterogeneity across single cells/nuclei are presented in Table 4. Several of the studies [54–60] combined single cell sequencing (SCS) with bulk tumor DNA sequencing, to refine descriptions of clonal architecture. The whole genome (or whole transcriptome) amplification necessary for SCS has the potential to cause technical errors, such as allelic dropout, amplification distortion, and false-positives [51,52]. Many different statistical methods have been developed to deconvolute SCS data to interpret cell lineages [52,61]. The degree to which the data analysis methods may impact the potential detection of multiclonal tumor origin is often unclear and an in-depth evaluation of such methods is beyond the scope of this review. Also, given the relatively small numbers of cells being sequenced, the sensitivity for detecting minor clonal populations is an issue. A common methodological approach used in SCS is to analyze single nucleotide polymorphisms/germline variants in normal tissue, which are subsequently filtered out as normal, even though these normal-appearing cells within tumors could be clones carrying invisible, epigenetic modifications. For these reasons, even though Hou et al. [62] concluded a monoclonal evolution pattern was most likely for JAK2-negative essential thrombocythemia samples, they acknowledged that early multiclonal tumor origin could not be precluded based on their SCS data. Of the 12 studies of single tumor cells described in Table 4, eight of them provide evidence supporting monoclonal tumor origin [including brain, breast, hematopoietic, and kidney tumors]. Four of the studies described in Table 4 provide evidence of multiclonal tumor origin [including tumors of the bladder, brain, breast, and colon].

8. Tumor lineage studies using aggregation chimeras and engineered rodents

Obtaining direct information about how one or more clones of cells drive the earliest events in tumor initiation is technically challenging and most approaches for assessing clonality have technical limitations. Fortunately, technologies are now available that can directly visualize cell lineage. The developmental history and state of the art regarding lineage tracing models have been described [63,64]. Lineage tracing models, including aggregation chimeras and engineered mouse models, have provided definitive data on the clonal origin of tumors.

Aggregation chimeras can be created by combining different early stage mouse embryos with each other or with pluripotent stem cells. They are useful tools because the component pluripotent stem cells and their progeny are phenotypically distinguishable. Mouse aggregation chimeras have been used to study the clonal origins of intestinal tumors [30]. Single embryos produced by a B6-Min (Min mice carry a truncating mutation in *Apc*) x B6-*ROSA26/ROSA26* cross (expresses β-galactosidase) were fused with single embryos produced by a B6 × B6-Min cross to generate intestinal tumor prone mice whose intestines contained patches of blue and white crypts after staining for β-galactosidase expression. This model is like studies of XCI in that it is possible to detect polyclonal tumor origin only for tumors that arise at sites where blue and white crypts co-localize. Because some of the intestinal adenomas that developed contained both blue and white sectors, the authors concluded the intestinal tumors were polyclonal in origin, with tumorigenesis driven by loss of the wild-type Min allele [30]. A second study incorporated a tumor resistance gene, *Mom1* into the embryos to reduce overall numbers of intestinal tumors and concluded that the excess number of blue/white tumors relative to what was expected based on blue/white patch sizes was consistent with short range clonal interactions driving intestinal tumorigenesis (rather than formation of collision tumors) [31]. This mouse model recapitulates the intestinal tumor-sensitivity of the germline condition, familial adenomatous polyposis (also due to *APC* loss).

More recently, a related model was used to study the clonality of intestinal tumors induced by somatic mutation. Specifically, ROSA26 aggregation chimeras were constructed on a background of *APC* wild-type B6 mice and subject to ethylnitrosourea-induced mutagenesis as adults [65]. Forty-seven percent of the induced tumors were overtly polyclonal, an increased polyclonal fraction compared to that observed in chimeric mice with the Min allele on both sides. Furthermore, an elementary stochastic model of clonal interaction via recruitment after initiation provided the best fit to the data on patch size and fraction of heterotypic tumors. The modeling predicted that clonal recruitment occurs over a distance of ~68 μm (95% CI, 38–121 μm), which is the approximate distance between the centers of two adjacent crypts [65]. Another study constructed aggregation chimeras using embryos from a strongly tumorigenic, $Apc^{Min/+}$ strain and a less tumorigenic, $Apc^{1638N/+}$ ROSA26 strain, as well as from a strongly tumorigenic, $Apc^{Min/+}$ strain and a wild-type, $Apc^{+/+}$ ROSA26 strain [66]. Again, modeling was used to evaluate whether clonal recruitment or clonal cooperation best explained the observed relationships between patch size and the

Table 2
Studies reporting detection of a single X chromosome-linked marker in non-neoplastic conditions, pre-neoplastic lesions, and tumors.[a]

Lesion or Tumor Type[b]	Method and X-linked Marker Examined	Number of Samples Analyzed	Reference
Non-neoplastic lesions			
Blood			
Paroxysmal nocturnal hemoglobinuria	RFLP/methylation of *AR*	5	[126]
Pre-neoplastic lesions			
Breast			
Ductal intraepithelial hyperplasia 1B	RFLP/methylation of *AR*	28	[41]
Liver			
High grade dysplastic nodules	RFLP/methylation of *AR*	24	[42]
Uterus			
Cervical intraepithelial neoplasia	RFLP/methylation of *AR*	3	[43]
Benign tumors			
Liver			
Hepatocellular adenoma	RFLP/methylation of *AR* and *PGK*	2	[46]
Lung			
Carcinoid	RFLP/methylation of *AR*	2	[132]
Pulmonary sclerosing hermangioma	RFLP/methylation of *AR* and *PGK*	22	[150]
Odontogenic tissue			
Ameloblastoma	RFLP/methylation of *AR*	5	[134]
Adenomatoid odontogenic tumor		2	
Calcifying odontogenic cyst		1	
Calcifying epithelial odontogenic tumor		1	
Ovary			
Ovarian-type mucinous tumors	RFLP/methylation of *AR*	9	[151]
Thymus			
Type AB thymoma	RFLP/methylation of *AR*	1	[152]
Uterus			
Benign metastasizing leiomyoma	RFLP/methylation of *AR*	1	[153]
Leiomyoma	RFLP/methylation of *AR or PGK*	315	[154]
adenomatoid tumor	RFLP/methylation of *AR*	10	[133]
Malignant tumors			
Bone Marrow			
Myelodysplastic syndrome (high risk)	RFLP/methylation of *AR* and SNP pyrosequencing	16	[26]
Myelodysplastic syndrome (CD34+, low risk)		13	
Myelodysplastic syndrome (CD34+, high risk)		9	
Breast			
Carcinoma in situ	RFLP/methylation of *AR*	10	[41]
Combined lobular and ductal carcinoma in situ	Methylation-specific PCR of *AR*	9	[155]
Cervix			
Adenocarcinoma	RFLP/methylation of *AR*	2	[128]
Gastrointestinal Tract			
Gastrointestinal stromal tumors	RFLP/methylation of *AR or PGK*	1	[156]
Kidney			
Clear cell renal cell carcinoma	RFLP/methylation of *AR*	8	[157]
Liver			
Hepatocellular carcinoma	RFLP/methylation of *AR* and *PGK*	4	[46]
Skin			
Kaposi sarcoma	RFLP/ methylation of *PGK*	5	[146]
Thymus			
Undifferentiated thymic carcinoma	RFLP/methylation of *AR*	1	[152]
Urinary tract			
Clear cell adenocarcinoma	RFLP/methylation of *AR*	2	[158] [159]
Urothelial carcinoma in situ		1	
Urothelial carcinoma		1	
Perivascular epithelioid cell neoplasm of the urinary bladder		1	
Uterus			
Adenosquamous carcinoma	RFLP/methylation of *AR*	6	[43]

AR, androgen receptor gene; *PGK*, phosphoglycerate kinase gene.
[a] Studies involving known hereditary cancer syndromes are not included.
[b] Tumors without classification as benign or malignant were placed in the benign tumor section of the table.

fraction of heterotypic tumors. The investigators concluded that recruitment is the mechanism driving the formation of polyclonal tumors and that recruitment may involve only a single neighboring cell within two or three crypts of the other progenitor clone.

While much has been learned using mouse aggregation chimeras, transgenic mouse models have been developed that have the advantages of conditional induction of tumor-initiating mutations and multiple lineage tracing fluorescent dyes, which provide better precision in the analysis of clonal interactions. These models have been used in conjunction with intravital microscopy to visualize interactions between different clones within lesions and how they change over time [67]. An $Apc^{+/-}$, transgenic mouse model was developed in which

Table 3
Genetic marker studies that provide information on the clonal origins of tumors.

Marker(s) Examined	Methods Used	Study Design	Major Conclusions Relevant to the Clonal Origins of Tumors	Reference
KRAS, *p53*, *p16* point mutations and microsatellite LOH for 16 loci on chromosomes 3p, 5q, 9p, 17p, 17q, and 18q.	Endoscopic mucosal resection specimens were serially sectioned and individual crypts isolated by laser capture microdissection	Conducted high resolution clonal-ordering within Barrett's lesions to produce phylogenetic trees and spatial maps of distinct clones in endoscopic mucosal resection specimens	Endoscopic mucosal resection specimens are genetically heterogeneous, individual crypts are clonal, but adjacent crypts have distinct mutations and LOH profiles	[160]
Presence of X/Y chromosomes in XO/XY tissue, 5q LOH, as well as *APC*, *p53*, and *KRAS* mutations	Fluorescence in situ hybridization, allele-specific PCR, and DNA sequencing	Examined microdissected crypts from small sporadic adenomas and sporadic carcinoma-in-adenomas	Colorectal microadenomas are multiclonal and independent clonal evolution can occur throughout adenoma development	[161]
Karyotype and CNV	Microdissected tumor cells sorted by ploidy then analyzed by representational oligonucleotide microarray (CGH) and FISH	Examined multiple sectors of breast cancers (sector-ploidy-profiling) to infer pathways of progression	Breast carcinomas display two classes of genomic structural variation: monogenomic and polygenomic (with extensive intermixing of subpopulations)	[162]
Mitochondrial mutations that result in cytochrome C oxidase deficiency, along with TP53 and APC mutations	Laser capture of cytochrome C oxidase-deficient crypts, followed by PCR sequencing	Examined the clonal nature of metaplastic crypts in the human stomach and compared to dysplasia	Intestinal metaplastic crypts are [mono]clonal, with single founder mutation within each dysplastic lesion	[163]
Mutations in *APC*, *CDKN2A*, *TP53*, *CTNNB1*, *KRAS*, *PIK3CA* and *PTEN*, along with LOH of chromosome 17	PCR sequencing and LOH analyses	Examined multiple regions of gastric adenocarcinomas	Cancer progression was initiated by an LOH event after which they become genetically diverse	[164]
B-cell translocation gene 1 (*BTG1*) deletions	Sensitive, breakpoint spanning PCR-based screening	Monitored precursor B-cell precursor ALL (BCP-ALL) patients and T-ALL patients over time	BCP-ALL patients carry multiple distinct *BTG1* deleted subclones that can expand clonally during relapse	[165]
Single nucleotide, indels, and copy number variants	Whole genome sequencing and array based gene expression and copy number profiling	Compared cell sorted myeloblasts from bone marrow of patients with secondary AML to the precursor disease, MDS in the same patients (skin as control)	Each myelodysplastic syndrome and secondarly AML genome contained a founding clone of cells consistent with monoclonal tumor origin.	[166]
Mutations in *TP53*, *CDKN2A* and *KRAS*	Crypt laser capture microdissection followed by PCR sequencing	Compared the mutational spectrum of early Barrett's adenocarcinomas with that of the surrounding dysplasia	Early Barrett's adenocarcinoma arises as a clonal outgrowth from multiclonal dysplasia	[167]
Single nucleotide variants	Whole genome sequencing combined with high depth re-sequencing to generate data on SNVs, CNV and LOH	Identified highly-mutated genes in esophageal adenocarcinoma (compared to normal), then compared benign metaplastic never dysplastic Barrett's esophagus with high grade dysplasia	The majority of genes recurrently mutated in esophageal adenocarcinoma are also mutated in never dysplastic Barrett's esophagus, and they occur in both tissues at similar variant frequencies	[168]
Somatic copy number alterations and somatic single nucleotide variants	Clonal Heterogeneity Analysis Tool (bioinformatic analysis pipeline)	Analyzed copy number and somatic variant data for normal and breast tumor DNA pairs from The Cancer Genome Atlas database	More than half of the 700 breast tumors examined contain multiple aneuploid tumor clones and many show sub-type specific differences in clonality for known cancer genes	[169]
Microsattelite markers	PCR and gel electrophoresis based analysis of microsattelites known to vary in hepatocellular carcinoma	Compared hepatocellular carcinoma with fibrosarcoma regions of hepatic carcinosarcoma	3/3 cases of hepatic carcinosarcoma are multiclonal in origin	[170]
Single nucleotide variants, copy number aberrations, and genomic rearrangements	Whole genome amplification, SNP microarray, quantitative PCR and FISH	Explored intrapatient and intratumoral heterogeneity of prostate cancers	Divergent tumor evolution and multiclonality observed in multfocal prostate cancers	[171]
Loss of hetero-zygosity in six polymorphic microsatellite markers	PCR amplification followed by polyacrylamide gel capillary electrophoresis	Compared normal and different areas of pleomorphic adenoma of the salivary gland	Intratumor heterogeneity in the form of loss of heterozygosity, with more heterogeneity in tumors < 2 cm in size compared to those > 2 cm in size	[172]
Single nucleotide variants, indels, CNVs, and analysis of a panel of 151 cancer genes	Whole exome sequencing and deep sequencing of cancer genes	Compared normal and multiple synchronous colorectal cancers from 20 different patients	Synchronous colorectal cancers have independent genetic origins, observed distinct clones in multiple tumors	[173]

(*continued on next page*)

Table 3 (*continued*)

Marker(s) Examined	Methods Used	Study Design	Major Conclusions Relevant to the Clonal Origins of Tumors	Reference
LOH of an APC mutational cluster region and high-sensitivity detection of *KRAS* mutations	High-depth NGS, SNP arrays, and high sensitivity genotyping of *APC* and *KRAS* by digital PCR	Analyzed 37 familial adenomatous polyposis colorectal adenomas, comparing normal, bulk lesions, and microdissected crypts	Multiple co-occurring truncal *APC* and *KRAS* mutations observed, consistent with a polycryptal model with multiple independent initiation lineages	[174]
Single nucleotide variants in *KRAS*, *BRAF*, and *PIK3CA*	Basescope, RNA in situ hybridization with sequencing for mutation confirmation	Created 2D maps of mutant and wild-type subclones in colorectal cancers	A complex clonal architecture exists in colorectal cancers	[175]

LOH, loss of heterozygosity; CNV, copy number variation; CGH, comparative genomic hybridization; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; SNV, single nucleotide variants; NGS, next generation sequencing.

intestinal cells fluoresce red unless they express Cre recombinase (controlled by the rat fatty acid binding protein I promoter), in which case the cells fluoresce green [68]. Fluorescence endoscopy and fluorescent microscopy of whole mount and sectioned tumors showed that between 43 and 68% of growths at different stages of tumorigenesis (from low grade dysplasia to invasive adenocarcinoma) had both red and green sectors, indicating a multi-ancestral architecture. A similar approach was used to show that 44% of tumors that arose in mice carrying the Min allele of *Apc* and expressing a constitutively active form of PI3K were derived from at least two ancestral clones [69].

Some studies employed "Confetti" mice for cell lineage tracing. In R26R-Confetti mice, once Cre recombination is induced, random rearrangement results in red, blue, green or yellow fluorescence, which is maintained in progeny cells. A study aimed at investigating cellular hierarchy in intestinal adenomas used a tamoxifen-inducible, confetti reporter and an Lgr5+ knock-in strain carrying a floxed Apc allele, which upon low dose tamoxifen injection causes *Apc* deletion and Lgr5+ intestinal stem cells to express randomly one of four fluorescent colors [70]. The large adenomas produced in this model either uniformly expressed a confetti color or had multiple independent segments expressing a different confetti color, consistent with the adenomas being derived from multiple independent stem cells.

A different multicolor lineage tracing mouse model was used to study the role of cancer stem cells in large and small intestinal tumors induced by 4-nitroquinoline-1-oxide [71]. In this gene-specific model system, Cre recombinase is fused to a mutated form of the ligand binding domain of the estrogen receptor and is inducible by tamoxifen in Bmi1-positive cells. Recombination results in the expression of one of four different fluorescent proteins that can be used to track clonal expansion. Using this model, it was determined that multiple single cells expanded clonally into units that in turn formed polyclonal tumors [71]. A similar model examined tumor cell lineages during intestinal tumorigenesis induced using three different approaches, 1) a genetic only approach using $Apc^{min/+}$ mice, 2) $Apc^{min/+}$ mice treated with dextran sodium sulfate, and 3) mice treated with azoxymethane and dextran sodium sulfate. The authors reported that the small sized colon tumors derived from Lgr5-positive cells were composed of cells labeled with a single color, whereas the large sized colon tumors were labeled with either a single color or multiple colors [72]. In the small intestine, both small and large tumors were composed of Lgr5-positive clones with different color fluorescence. Interpretation of these results is complicated by the fact that only cells expressing specific stem cell markers were assessed and that tumorigenesis occurred before the lineage tracing expression of fluorescent markers was initiated.

A study of DMBA-induced mouse skin papillomas in confetti mice expressing $Hras^{Q61L}$ supported the earlier conclusion of Thliveris and coauthors, that individual clones within the mouse intestine could recruit a second discrete clone with a range of 144 μm, by facilitating its transformation [66]. At 12 weeks, 78% of papillomas in the skin of DMBA-treated mice are singly-colored and 22% have a second color, but by 20 weeks 78% have incorporated one or more secondary population(s), identified as streaks of distinct color [73]. Because the secondary populations do not carry the $Hras^{Q61L}$ mutation, the papillomas appear to recruit pseudo-normal clones from their microenvironment early in tumor development.

The clonal architecture of pancreatic cancer has been studied using confetti mice. In a confetti mouse model that expressed oncogenic $Kras^{G12D}$ in the context of a single *p53* allele deletion, it was found that 24% of acinar to ductal metaplasias were polychromatic, indicating they arose from multiple distinct acinar cells [74]. Because 97% of pancreatic intraepithelial neoplasias were monochromatic, clonal diversity was lost relatively quickly during progression. Importantly, this study also observed that nearly 80% of well-separated, metastases to the peritoneal wall or diaphragm were biclonal, with lower percentages of biclonal metastases observed in liver and lung [74]. Bichromatic cellular clusters were observed in ascites fluid of tumor-bearing mice.

Table 4
Single cell studies that address the clonal origins of tumors.

Marker(s) Examined	Study Design	Major Conclusions Relevant to the Clonal Origins of Tumors	Reference
Copy number	Flow sorted nuclei (100 and 52) from multiple sectors of two triple negative ductal carcinomas and a liver metastasis of one (48 nuclei) were sequenced.	Clear clonal relationships were observed among aneuploidy cell populations that reflect punctate evolution, with a substantial portion of the tumor mass comprised of pseudodiploid cells distinct from the highly clonal tumor subpopulations, consistent with multiclonal tumor origin.	[176]
Somatic mutation	Exome sequences from 20 cells of a clear cell renal cell carcinoma were compared to five cells from normal tissue.	The tumor did not contain any significant clonal subpopulations.	[177]
Somatic mutation	Sequences from 44 cells of a muscle-invasive bladder transitional cell carcinoma were compared to 11 cells from normal adjacent tissue.	The data was consistent with the muscle-invasive bladder transitional cell carcinoma being monoclonal in origin, with evolution leading to two distinct tumor cell subpopulations.	[178]
Somatic point mutations	Sequences of cells (58) from bone marrow of a JAK2-negative essential thrombocythemia patient were compared with cells from oral mucosa.	The evidence indicated a monoclonal evolution pattern was most likely for the JAK2-negative essential thrombocythemia, but the authors stated a brief polyclonal stage, where a specific clone had a very strong growth advantage, could not be ruled out.	[62]
Analyzed a specific gene fusion, along with specific nucleotide and copy number variants	Multiplex Q-PCR from variant detection was conducted on flow-sorted single cells from bone marrow samples of a Down's syndrome ALL (115 cells) and two ETV6-RUNX1 positive ALLs (261 and 254 cells), previously analyzed by bulk DNA sequencing.	Reconstructed, branching architectures were consistent with monoclonal tumor origin.	[54]
Single nucleotide and copy number variants	Exome sequences from single cells (63) of a colonic adenocarcinoma were compared with that of normal tissue and bulk tumor tissue.	Colon cancer contained a major clonal population carrying *APC* and *TP53* mutations and a minor clone lacking these mutations, indicating a biclonal tumor origin	[56]
Single nucleotide and copy number variants	Whole exome sequences of flow-sorted, aneuploid G2/M nuclei (47) from an ER + /PR + breast cancer were compared to that of matched normal tissue, while 50 aneuploid G2/M nuclei were used for copy number profiling. Similar approaches were used to analyze hypodiploid, diploid and aneuploid G2/M tumor cells of a triple-negative invasive ductal carcinoma.	Reconstructed architectures consistent with monoclonal tumor origin. Chromosome rearrangements occurred early, in punctuated burst of evolution followed by clonal expansion. The TNBC exhibited a higher mutation rate than the ER positive breast cancer.	[55]
Single nucleotide variants, deletions and IgH sequence diversity	Targeted sequencing of regions of genomic heterogeneity previously identified by bulk tumor sequencing of bone marrow (compared to saliva samples) from six children with B-ALL (a total of 1479 ALL cells).	Identified co-dominant clones consistent with monoclonal tumor origin. Most large deletions occur before cytosine mutagenesis-driven single nucleotide variant acquisition, with VDJ recombination occurring throughout clonal evolution.	[58]
Somatic mutation, *EGFR* structural and copy number variants	Flow-sorted nuclei from two *EGFR*-amplified primary glioblastomas (previously examined by bulk sequencing) were analyzed using 50–60 cells, compared to matched blood DNA.	*EGFR* truncation variants identified in the bulk tumor sequencing segregated into non-overlapping subclonal populations. But, in both glioblastomas, the EGFR mutant subpopulations possessed a shared truncal mutation, consistent with monoclonal tumor origin.	[57]
Single nucleotide variants	Used hybridization capture for targeted analyses of three subjects (cells) with secondary AML, using normal skin as a control. Analyzed heterogeneity previously detected by bulk tumor DNA sequencing.	Single cell sequencing demonstrated distinct cell populations arising at successive points in tumor evolution, consistent with monoclonal tumor origin.	[60]
Gene expression profile and copy number variants	Five *IDH1/2* wild-type primary glioblastomas were analyzed by single cell RNA sequencing (96–192 cells per tumor) and compared to normal brain.	Identified a minor population of tumor cells with CNV patterns similar to normal oligodendrocytes and observed in the majority of the tumor cells, consistent with multiclonal tumor origin.	[179]
Copy number variants	Whole genome sequences from flow-sorted diploid nuclei of two ER + breast cancers were analyzed (using 86 and 89 cells).	Identified subclonal variations and mosaicism consistent with monoclonal tumor origin.	[59]
PIK3CA H1047R mutation and *HER2* (*ERBB2*) amplification	Used specific-to-allele PCR-FISH (STAR-FISH) to visualize *PIK3CA* mutant and wild-type sequences, and *ERBB2* amplification in situ in individual cells of HER2 positive breast tumor samples before and after neoadjuvant treatment.	*PIK3CA* H1047R mutation and HER2 (*ERBB2*) amplification are not always present in the same cells and neoadjuvant treatment selects for minor subpopulations of *PIK3CA* mutant cells	[53]

ALL, acute lymphoblastic leukemia; ER, estrogen receptor; PR, progesterone receptor; TNBC, triple-negative breast cancer; CNV, copy number variation.

When isolated clusters and single cells were injected into the peritoneal cavity of immunocompromised mice (NOD SCID), bichromatic clusters produced a greater metastatic burden (in the diaphragm or lung) than isolated single cells [74]. A second study using confetti mice found early monoclonal and polyclonal lesions, including acinar to ductal metaplasia and early and late pancreatic intraepithelial neoplasias [75].

Studies of confetti mice have some caveats that may impact the detection of multiclonality. Not all areas of a tissue sample display fluorescence and a single fluorescent color may be overrepresented, reducing the potential to discern multiclonality. Nevertheless, this is a powerful technology for identifying clonal interactions, one that is likely to yield additional important information regarding the earliest events in tumorigenesis. Going forward, results obtained using lineage tracing mouse models that conditionally alter the expression of cancer-associated genes will be valuable because such models reflect accurately the stochastic somatic events involved in human tumor development [76].

9. Integrating multiclonal tumor origin with theories of tumorigenesis

Monoclonal tumor origin and multi-stage carcinogenesis have dominated theories of carcinogenesis, with their acceptance shaping clinical oncology and regulatory risk assessment practices [e.g., provided the rationale for using monotherapies in personalized cancer treatment and the linear no-threshold (LNT) model for cancer risk assessment]. However, the explosion of data generated using massively parallel sequencing has created an opportunity to reconsider and refine current understanding of carcinogenesis. In the following section, recent observations regarding specific aspects of tumorigenesis (with emphasis on early events) are discussed in terms of their compatibility with multiclonal tumor origin.

Field cancerization can be viewed either as the earliest stage of tumorigenesis or as a step preceding tumorigenesis. The term was used by Slaughter and colleagues in 1953 to explain the prevalence of multiple oral cancers; 'epidermoid carcinoma of the oral stratified squamous epithelium originates by a process of "field cancerization," in which an area of epithelium has been preconditioned by an as-yet-unknown carcinogenic agent' [77]. More recently, Lochhead et al. [78] proposed an 'expanded, integrative concept, "etiologic field effect", which asserts that various etiologic factors (the exposome including dietary, lifestyle, environmental, microbial, hormonal, and genetic factors) and their interactions (the interactome) contribute to a tissue microenvironmental milieu that constitutes a "field of susceptibility" to neoplasia initiation, evolution, and progression.' This view of the etiologic field effect encompasses multiple cell types of different developmental lineages and epigenetic, as well as genetic, lesions [77,79,80]. Although the clonal expansion associated with field cancerization is described as arising from a single progenitor cell [10,81,82], the concept of field cancerization is easily reconciled with multiclonal tumor initiation. Specifically, the prevalence of mutants in normal tissues [83–86] and evidence that a mutant cell or cell type may drive the transformation of another (discussed below) provides a foundation for linking field cancerization and multiclonal tumor origin.

Brash [50] summarizes a large body of work characterizing the presence of mutant cells in normal tissues. Data derived from 12 different tissue types shows that mutations capable of contributing to the carcinogenic process are present in many normal tissues, including sun-exposed skin, esophagus, colon, breast, pancreas and lung [10,50]. Field cancerization can be equated with initiation when tumorigenesis follows. However, for any particular cell or group of cells mutated as a part of field cancerization, clonal expansion and tumorigenesis may not occur. Brash [50] proposed that clones of mutant cells in normal tissues be described as "proto-cancer," because not all clones will progress to cancer and progression requires additional events. In man, many different types of field effect markers have been identified [77], and fields of pro-cancerous mutations have been associated with cancer-predisposing diseases, such as ulcerative colitis, Crohn's disease and Barrett's esophagus [47,50,79]. In a conditional Apc knock-out mouse model (Lgr5-CreER), where the sizes of Apc deficient clones within the intestine were manipulated through the tamoxifen dose used to induce Cre recombination, it was observed that the Apc deficient field size correlated positively with the degree of adenoma formation [76]. When this experiment was performed on a "confetti" background, it was found that the induced adenomas were multiclonal [76].

The nature and number of at risk cells and cell types are critical issues in terms of modeling tumorigenesis. According to the cancer stem cell hypothesis, only specific cells with self-renewing capabilities can drive tumorigenesis because these are the only cells retained long enough to undergo the additional steps necessary for tumorigenesis [87]. According to the cancer stem cell hypothesis, differentiation of initiated stem cells produces the multiple cell types within a tumor of monoclonal tumor origin [87,88]. Such tumor cell plasticity is supported by evidence of epithelial to mesenchymal and mesenchymal to epithelial transitions [89]. As the cancer stem cell hypothesis is generally understood, it is not compatible with multiclonal tumor origin. However, an interaction between more than one pluripotent stem cell, leading to tumor initiation, is a potential path to multiclonal tumor origin.

An alternative theory regarding tumor cell(s) of origin is that oncogenic transformation causes differentiated cells to become cancer stem cell-like cells [90]. Some evidence for this comes from a *BRAF* V600E-driven mouse model of melanoma in which mouse cutaneous melanomas arose from expansion and dedifferentiation of mature pigmented melanocytes [91]. The literature includes other more nuanced views of which cells are at risk for becoming tumor cells of origin. Oncogenic transformation can be viewed as disrupting normal cell differentiation hierarchies [80]. Graham and Wright [92] describe a complex picture related to the gastrointestinal stem cell as the cancer cell of origin. They suggest that the stem cell phenotype is a combination of the niche (including the extrinsic signaling properties determined by the cells around the niche) and the intrinsic properties of the cell within the stem cell niche (which can also be altered through a process of niche succession) [79]. In the context of epidermal proliferating units of the skin, Brash [50] describes how pro-cancerous cells may undergo clonal expansion by altering the frequencies with which committed progenitor (CP) cells divide to give two CP cells, to give one CP and one post-mitotic cell, or to give two post-mitotic cells [50]. In human skin, clonal expansion is linear, not exponential and not all clones progress, some are eliminated while some grow more slowly than others [50]. Multiclonal tumor origin can be reconciled with these views of at-risk cells, by postulating that tumor cells of origin carry mutations or epigenetic changes within stem cells, differentiated epithelial cells and/or stromal cell clones that cooperate to alter CP cell fate [88].

Multiclonal tumor origin is both consistent with and supported by evidence of clonal cooperativity [47,79,93], although clonal cooperativity may also occur between clones of cells sharing a truncal mutation (see Fig. 2A). Cooperativity between epithelial, stromal, and immune cells is a well-described phenomenon in tumor progression [79,93–96]. Clonal cooperation is depicted in Fig. 4. The hypothesis that a mutated epithelial clone with altered signaling affects nearby stroma such that the altered stroma drives mutagenesis in surrounding epithelia has been offered as an explanation for how field cancerization results in polyclonal tumor development [79]. But, the possibility also exists that aberrant stroma drives tumorigenesis. Per the tissue organization field theory, "abnormal interactions between mesenchyme/stroma and the parenchyma" are the basis of tumor development [97] and abnormal communication between stroma and epithelia could arise from genetic and/or epigenetic alterations in either or both compartments. Cooperation between an epithelial cell carrying a genetic lesion with an activated fibroblast has been proposed for oral cancer [96]. Clonal cooperativity was demonstrated in drosophila eye-antennal discs, where clones overexpressing mutant Ras^{V12} overgrow moderately, clones deficient in the tumor suppressor *scribbled* die, but when the two clones are adjacent large metastatic tumors develop through a mechanism that involves JNK-induced upregulation of JAK/STAT-activating cytokines [98]. Similar cooperative clonal growth between distinct clones has been observed in a rodent model of human breast cancer (cooperation between *Hras* mutant basal clones and WT *Hras* luminal clones), in a rodent model of human pancreatic cancer (cooperation between *Kras* and *Tp53* mutant clones), and in primary human glioblastoma (cooperation between EGFR mutant and WT clones), among others [50,99–101].

Clonal cooperation may be necessary to overcome normal tissue homeostatic mechanisms [50,102]. When cancer driver mutations detected in "proto-cancers" are expressed constitutively in rodents, they induce tumors. But the growth of the same mutations in normal epithelium is restricted [50,81,103]. Cell mixing studies that describe the fraction of normal cells needed to hold oncogene activated cells in check have been reviewed [50]. Paracrine signaling is a potential

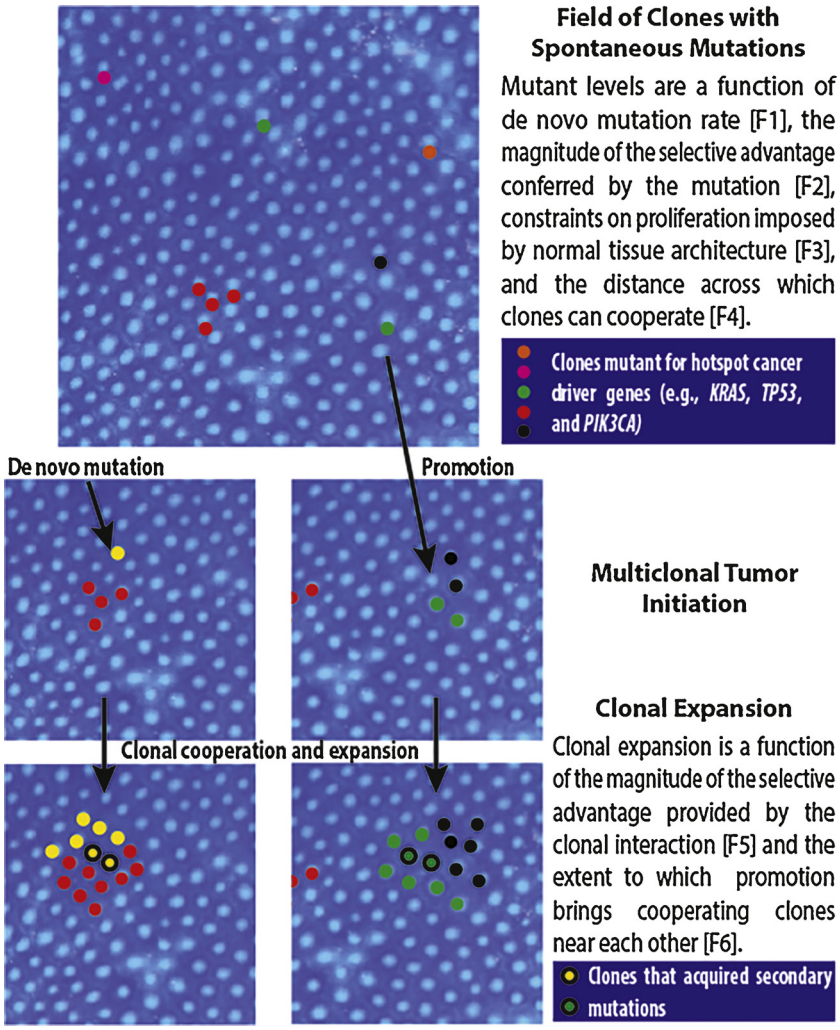

Fig. 4. Clonal cooperation and factors [F#] that should be considered in biologically-based mathematical models of multiclonal tumorigenesis are presented. A field of clones carrying spontaneous mutations (colored dots) is depicted (top panel). Factors that contribute to the resulting field effect in terms of pro-cancer mutations within normal tissues include: Factor 1, the frequency of de novo mutation (a consequence of intrinsic mutagenesis); Factor 2, the selective advantage of mutant cells, which may drive clonal expansion (red clone); Factor 3, the competing effect of resistance to clonal expansion conferred by the tissue hierarchy and surrounding wild-type cells, and Factor 4, the distance across which clones can cooperate. Multiclonal tumor initiation could occur by de novo mutation (either intrinsically or extrinsically induced) in cells adjacent to a mutant clone (see middle left panel, "de novo mutation," yellow clone). This may occur at a greater frequency than secondary mutation in the subset of already mutant cells (as is required for monoclonal tumor initiation) and result in clonal expansion. Other factors expected to impact clonal expansion are Factor 5, the selective advantage produced by clonal cooperation and Factor 6, the extent to which promotion due to intrinsic (e.g., epigenetic) and extrinsic causes could result in clonal expansion, placing cooperating clones within sufficient distance to confer a cooperative selective advantage (bottom right panel, green and black clones).

mediator of clonal cooperation [93,98,100,104], suggesting that the distance between interacting clones may be an important determinant in tumor progression [65,66]. As discussed previously, multiple studies provide evidence of short-range clonal interactions, interactions between mutant and pseudonormal clones, and interactions involving clonal migration, as early events in tumorigenesis [31,47,73,79]. Thus, the idea that field cancerization provides the substrate (proto-cancer) for clonal interactions, which in turn drive tumor initiation/progression, is entirely consistent with multiclonal tumor origin, as are observations indicating early clonal expansion is stochastic in nature with different lesions having different fates, i.e., some progress whereas others remain static or even regress [50,105,106].

Sufficient evidence of tumor subclonal interactions has come to light that tumors are being described as cancer ecosystems that should be modeled using approaches developed for complex ecosystems or microbial consortia [93–95,107]. After describing evidence of multiclonal precancerous lesions, Brash writes "polyclonality of precancerous lesions – at least in epithelial tissues – opens two possibilities: (a) they are societies of cooperating clones and (b) selection on this society follows the laws of metapopulation dynamics, a field of evolutionary theory that deals with populations composed of interacting subpopulations" [50].

An approach to understanding the carcinogenic process has been to reconcile the mathematics surrounding the biology of tumor development with tumor incidence data. Conducting such analyses requires making critical assumptions about mutation rate, number of at-risk cells, number of events required for tumorigenesis, and how these should relate to each other mathematically. Factors rarely integrated into such models are: 1) the selective advantage and consequent expansion of cells carrying pro-cancerous mutations, 2) the impact of clonal cooperativity between clones within a "pre-cancerous field," 3) the potential for cooperating clones to move toward each other within a tissue, and 4) multiclonal tumor origin [108]. It is sometimes suggested that the frequency of oncogenic mutations makes it improbable that tumors can be multiclonal in origin (see [109]). However, this argument is based on the "classic" interpretation that multiple mutations occur sequentially within a single cell lineage as a nascent tumor progresses toward malignancy [110]. More recent analyses of mutations and copy number alterations are not consistent with sequential and gradual clonal evolution. Based on genomic profiling of 349 individual glands from 15 colorectal tumors, Sottoriva et al. [111] proposed a "Big Bang" model of colorectal tumor growth. According to this model, "after the initial transformation, colorectal tumors grow predominantly as a single expansion populated by numerous intermixed subclones [111]. Evidence has been reported supporting other types of clonal evolution, including linear, branching, neutral, and punctuated equilibrium [82,112]. Sottorvina et al. [111] described the Big Bang model in the context of monoclonal tumor origin. However, Sievers et al. [113] reported multiclonal colon polyps had a mutational profile consistent with the Big Bang model of tumorignenesis and/or with polyps arising from a field of genetically heterogeneous cells [105]. Gao et al. [114] sequenced 1000 single cells from 12 triple-negative breast cancers and concluded most tumors were composed of one to three major clonal subpopulations with a common lineage and a minor subpopulation of

non-clonal cells. In addition, they concluded that the copy number aberrations were acquired at the earliest stages of tumor development in short punctuated bursts followed by stable clonal expansion [114]. Thus, it appears that more precise information regarding the early stages of tumorigenesis is leading away from theories of clonal tumor development that involve stepwise accumulation of mutation within a single clonally expanding population of monoclonal origin toward contemporaneous interaction between multiple, spatially proximate clones, a view consistent with multiclonal tumor origin [115].

Tumor evolution, as a whole, is the consequence of selection at the level of the individual cell [102]. Factors that drive expansion of nascent clones include oncogenic mutations that confer a selective advantage and clonal interactions. The selective advantage of mutant cells is one aspect of tumorigenesis that has generally been omitted from mathematical models, probably due to the dearth of useful information. Nevertheless, the selective advantage conferred by a *KRAS* G12D mutation in human colon was recently estimated [116]. Mathematical approaches are being explored that incorporate microenvironment- and age-dependent cellular fitness of cells carrying somatic mutations into tissue evolutionary models of cancer [108]. Tomlinson [117] has proposed that it is unnecessary to invoke a mutator phenotype to explain the large number of mutations that accumulate within tumors if one assumes that mutations increase the fitness of mutant cells during the sequential accumulation of mutations.

The number of pro-cancerous cells within normal tissues is an additional factor that should be incorporated into mathematical frameworks describing tumor development. The prevalence of oncomutation in normal human tissues can be remarkably high [83–86]. For example, *RAS* and *PIK3CA* mutant cells, are prevalent [118] and present at relatively high frequency (10^{-5}–10^{-1}) in multiple normal tissues of healthy individuals [84]. Importantly, the variability in hotspot *KRAS* and *PIK3CA* mutant levels within particular tissues correlated strongly with the prevalence of the corresponding mutation in tissue-specific carcinogenesis [84]. This supports the idea that variability across individual normal samples provides a measure of clonal expansion/selective advantage for specific tissue type/mutation combinations. A remarkably high frequency of cancer driver mutations was observed by ultradeep sequencing of 234 biopsies across normal sun-exposed eyelid skin (140 driver mutations per cm^2), which was attributed to multiple cancer genes under strong positive selection [119]. This density of events increases the likelihood that multiclonal cooperativity is a frequent early event in tumorigenesis.

There is controversy regarding the extent to which endogenous and exogenous processes contribute to the human cancer burden, as interpreted by mathematical modeling [109,120–123]. Currently, there are too many carcinogenic factors that are not parameterized in these models to make this a fruitful discussion. At least six different factors that are generally not parameterized in mathematical models of carcinogenesis are depicted in Fig. 4. Biologically-based mathematical models of carcinogenesis are needed that incorporate the proximity and frequency of cooperating lesions, the positive and negative selective pressures conferred by mutations (singly and in combination), and the impact of cell division hierarchy within a tissue, along with the potential contributions of paracrine signaling and tumor cell migration. It seems likely there will be different predominant paths to carcinogenesis for different tissues and multiple pathways to carcinogenesis within a given tissue. Thus, to be meaningful, mathematical models of carcinogenesis need to incorporate more biological aspects/parameters of tumor initiation and progression, particularly as they relate to the probability of multiclonal tumor origin.

10. Conclusions and implications

Evidence of multiclonal tumor origin continues to accumulate. Although the term polyclonal has been used most often to express other than monoclonal tumor origin, the term multiclonal is probably a better choice, because most studies describing multi-lineage lesions/growths report two or a few founding clones. For some cancer types, like colorectal cancer, a large body of evidence supports multiclonal tumor origin as the predominant mode of tumorigenesis. For tumors of myeloid cell lineages, most (but not all) evidence supports monoclonal tumor origin. Studies summarized here (combined data from Tables 1, 3 and 4 and [1]) support multiclonal origin for at least some portion of 53 different tumors that develop across 24 different anatomical sites (see Supporting information, Table S1). When these data are extrapolated, taking into account the technical biases against detecting multiclonality and the potential for pseudomonoclonality in fully-developed tumors, it seems likely that most tumors are multiclonal. Different cell types (e.g., epithelial, stromal and immune) clearly interact during tumor initiation and progression [93]. And, there is a large and growing body of evidence regarding cooperation and/or recruitment between cells carrying different genetic lesions.

There has been a reticence within the scientific community to recognize multiclonal tumor origin as a major path to carcinogenesis. Monoclonality is still considered a defining characteristic of neoplasia. While it is true that multiclonality is detected more often in early tumors than in fully-developed malignancies, interpretation of multiclonality (i.e., a non-monoclonal growth) as counter to a finding of neoplasia is not justified. Oncology training and medical texts should recognize that pre-neoplastic and neoplastic lesions can be multiclonal and that multiclonal preneoplastic lesions often develop into pseudomonoclonal neoplastic growths.

Multiclonal tumor origin has important implications for cancer risk assessment and chemical safety assessment. For many tissues, carcinogen-driven step-wise accumulation of mutations within the lineage of a single cell may be minimal compared to the frequency of carcinogen-induced mutant clones interacting with fields of spontaneously induced and clonally-expanded mutant cell populations. If true, then spontaneous mutations that confer a selective advantage (i.e., hotspot cancer driver mutations) are substrates for chemical carcinogenesis and, as such, will be useful reporters for assessing the potency of carcinogens (including tumor promoters) [83]. Regulatory bodies currently employ the linear-no-threshold (LNT) model in the cancer risk assessment of ionizing radiation and mutagenic chemical carcinogens, whereas tumor promoters may be considered to have a threshold [124]. The appropriateness of this paradigm should be reassessed, taking into account current knowledge regarding the complexity of the mechanisms underlying tumor development. Given the abundance of such proto-cancer cells in normal tissues, should mutagens always be considered as posing a greater cancer risk than tumor promoters? And, what is the relative risk of chemicals that impact the epigenome?

The most important implications regarding multiclonal tumor origin relate to how researchers and clinicians approach cancer therapy. It is now known that small populations of pre-existing, founder clones are maintained within tumors and contribute to therapeutic resistance, particularly to molecularly-targeted, monotherapies [4]. The scope of this problem is much larger than has been recognized. It seems likely that a large majority of tumors carry minor mutant subpopulations of founder clones, which do not possess the genetic lesion(s) present in the bulk of the tumor. These subpopulations may be small enough to make them undetectable in the clinical setting but nevertheless are sufficient to drive therapeutic resistance and patient relapse. New ideas and more sweeping approaches are needed to address this issue. If it were known which genetic lesions were present in the founder clones that most frequently cooperate with the founder lesions detected in patients' fully-evolved tumors, then useful combination therapies might be envisioned and evaluated. Founder clones will vary across individual tumors and across different tumor types, but the repertoire of genetic lesions present in founder clone combinations may be small, compared to the repertoire of genetic lesions present in fully developed tumors. In the future, molecularly targeted therapies that deplete the fields of pro-cancer cells may be used to prevent cancer. In any case, the first step

toward rationally addressing the issue of resistance to cancer therapy is recognizing that most tumors may well have multiple founder clones.

Conflict of interest statement

None.

Acknowledgements

The authors thank Dr. Kelly L. Harris and Dr. Robert H. Heflich for their helpful review of the manuscript. Tissue samples were provided by the Cooperative Human Tissue Network which is funded by the National Cancer Institute. Other investigators may have received specimens from the same subjects. The information in these materials is not a formal dissemination of information by FDA and does not represent agency position or policy.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mrrev.2018.05.001.

References

[1] B.L. Parsons, Many different tumor types have polyclonal tumor origin: evidence and implications, Mutat. Res. - Rev. Mutat. Res. 659 (2008) 232–247.
[2] NIH, NIH (Ed.), Cell Biology and Cancer, NIH, Washington, DC, 1999, https://science.education.nih.gov.
[3] L.A. Diaz Jr., R.T. Williams, J. Wu, I. Kinde, J.R. Hecht, J. Berlin, B. Allen, I. Bozic, J.G. Reiter, M.A. Nowak, K.W. Kinzler, K.S. Oliner, B. Vogelstein, The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers, Nature 486 (2012) 537–540.
[4] B.L. Parsons, M.B. Myers, KRAS mutant tumor subpopulations can subvert durable responses to personalized cancer treatments, Pers. Med. 10 (2013) 191–199.
[5] J.C.Y. Wang, J.E. Dick, Cancer stem cells, in: V.T. DeVita, T.S. Lawrence, S.A. Rosenberg (Eds.), Cancer: Principles & Practice of Oncology: Primer of the Molecular Biology of Cancer, Lippincott Williams & Wilkins, Philadelphia, PA, 2011, pp. 128–140.
[6] S. Dhingra, D. Tan, Cancer stem cells, in: D. Tan, H.T. Lynch (Eds.), Principles of Molelcular Diagnostics and Personalized Cancer Medicine, Lippincott Williams & Wilkins, Philadelphia, PA, 2013pp. 12–28.
[7] P.C. Nowell, The clonal evolution of tumor cell populations, Science 194 (1976) 23–28.
[8] W.C. Hahn, R.A. Weinberg, Cancer: a genetic disorder, in: J. Mendelsohn (Ed.), The Molecular Basis of Cancer, Elsevier Saunders, Philadelphia, AP, 2015pp. 3–17.
[9] R.A. Weinberg, The nature of cancer, The Biology of Cancer, Garland Science, Taylor & Francis Group, LLC, New York, NY, 2014 pp. 31–70.
[10] S.B. Garcia, H.S. Park, M. Novelli, N.A. Wright, Field cancerization, clonality, and epithelial stem cells: the spread of mutated clones in epithelial sheets, J. Pathol. 187 (1999) 61–81.
[11] A.R. Black, K.H. Cowan, Cancer biology and genetics, in: L. Goldman, A.I. Schafer (Eds.), Goldman-Cecil Medicine, Elsevier Saunders, Philadelphia, PA, 2016.
[12] NCI, Understanding Cancer, What Is Cancer, NIH, Rockville, MD, 2015https://www.cancer.gov/about-cancer/understanding/what-is-cancer#how-cancer-arises.
[13] C.S.O. Attolini, Y.K. Cheng, R. Beroukhim, G. Getz, O. Abdel-Wahab, R.L. Levine, I.K. Mellinghoff, F. Michor, A mathematical framework to determine the temporal sequence of somatic genetic events in cancer, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 17604–17609.
[14] B.L. Parsons, Monoclonal tumor origin is an underlying misconception of the RESIC approach, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) E15.
[15] Y.K. Cheng, R. Beroukhim, R.L. Levine, I.K. Mellinghoff, F. Michor, Reply to Parsons: many tumor types follow the monoclonal model of tumor initiation, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) E16.
[16] D. Ramazzotti, A. Graudenzi, L. De Sano, M. Antoniotti, G. Caravagna, Learning mutational graphs of individual tumor evolution from multi-sample sequencing data, bioRxiv (2017), http://dx.doi.org/10.1101/132183.
[17] L. Cheng, S. Zhang, G.T. MacLennan, S.R. Williamson, D.D. Davidson, M. Wang, T.D. Jones, A. Lopez-Beltran, R. Montironi, Laser-assisted microdissection in translational research: theory, technical considerations, and future applications, Appl. Immunohistochem. Mol. Morphol. 21 (2013) 31–47.
[18] N. Agarwal, R.H. Nussenzveig, S.I. Swierczek, C.J. Parker, J.T. Prchal, Does HUMARA assay for assessment of clonal hematopoiesis have shortcomings? Blood 114 (2009) 2357–2358.
[19] G.L. Chen, J.T. Prchal, X-Linked clonality testing: interpretation and limitations, Blood 110 (2007) 1411–1419.
[20] J.R. Jones, Nonrandom x chromosome inactivation detection, Current Protocols in Human Genetics, (2014) Unit 9.7.
[21] A. Hellman, A. Chess, Gene body-specific methylation on the active X chromosome, Science 315 (2007) 1141–1143.
[22] L. Carrel, H.F. Willard, X-Inactivation profile reveals extensive variability in X-linked gene expression in females, Nature 434 (2005) 400–404.
[23] R. Chaligné, E. Heard, X-Chromosome inactivation in development and cancer, FEBS Lett. 588 (2014) 2514–2522.
[24] S.J. Leedham, N.A. Wright, Human tumour clonality assessment–flawed but necessary, J. Pathol. 215 (2008) 351–354.
[25] J. Wang, R. Yu, S. Shete, X-Chromosome genetic association test accounting for X-inactivation, skewed X-inactivation, and escape from X-inactivation, Genet. Epidemiol. 38 (2014) 483–493.
[26] M. Mossner, D. Nowak, M. Klaumünzer, V. Nowak, K. Ackermann, S. Brendel, J. Obländer, O. Hopfer, C. Baldus, E. Thiel, W.K. Hofmann, Detection of highly clonal CD34+, mononuclear bone marrow and peripheral blood cells from patients with myelodysplastic syndrome and age related increase of clonal hematopoiesis in healthy subjects using a novel transcriptional pyrosequencing based clonality assay, Blood 116 (2010) 977.
[27] G.P.S. Knudsen, J. Pedersen, O. Klingenberg, I. Lygren, K.H. Ørstavik, Increased skewing of X chromosome inactivation with age in both blood and buccal cells, Cytogenet. Genome Res. 116 (2007) 24–28.
[28] S.I. Swierczek, N. Agarwal, R.H. Nussenzveig, G. Rothstein, A. Wilson, A. Artz, J.T. Prchal, Hematopoiesis is not clonal in healthy elderly women, Blood 112 (2008) 3186–3193.
[29] M. Novelli, A. Cossu, D. Oukrif, A. Quaglia, S. Lakhani, R. Poulsom, P. Sasieni, P. Carta, M. Contini, A. Pasca, G. Palmieri, W. Bodmer, F. Tanda, N. Wright, X-inactivation patch size in human female tissue confounds the assessment of tumor clonality, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 3311–3314.
[30] A.J. Merritt, K.A. Gould, W.F. Dove, Polyclonal structure of intestinal adenomas in Apc+ min/+ mice with concomitant loss of Apc++ from all tumor lineages, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 13927–13931.
[31] A.T. Thliveris, R.B. Halberg, L. Clipson, W.F. Dove, L.M. Sullivan, M.K. Washington, S. Stanhope, M.A. Newton, Polyclonality of familial murine adenomas: analyses of mouse chimeras with low tumor multiplicity suggest short-range interactions, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6960–6965.
[32] M.R. Novelli, J.A. Williamson, I.P. Tomlinson, G. Elia, S.V. Hodgson, I.C. Talbot, W.F. Bodmer, N.A. Wright, Polyclonal origin of colonic adenomas in an XO/XY patient with FAP, Science 272 (1996) 1187–1190.
[33] L. Jovanovic, B. Delahunt, B. McIver, N.L. Eberhardt, S.K.G. Grebe, Thyroid gland clonality revisited: the embryonal patch size of the normal human thyroid gland is very large, suggesting X-chromosome inactivation tumor clonality studies of thyroid tumors have to be interpreted with caution, J. Clin. Endocrinol. Metab. 88 (2003) 3284–3291.
[34] G.W. Moore, J.J. Berman, Cell growth simulations predicting polyclonal origins for' monoclonal' tumors, Cancer Lett. 60 (1991) 113–119.
[35] L. Pozo-Garcia, S.J. Diaz-Cano, B. Taback, D.S.B. Hoon, Clonal origin and expansions in neoplasms: biologic and technical aspects must be considered together [1] (multiple letters), Am. J. Pathol. 162 (2003) 353–355.
[36] A. Levy, Monoclonality of endocrine tumours: what does it mean? Trends Endocrinol. Metab. 12 (2001) 301–307.
[37] S. Swierczek, L.T. Lima, T. Tashi, S.J. Kim, X.T. Gregg, J.T. Prchal, Presence of polyclonal hematopoiesis in females with Ph-negative myeloproliferative neoplasms, Leukemia 29 (2015) 2432–2434.
[38] D. Musalkova, J. Minks, G. Storkanova, L. Dvorakova, M. Hrebicek, Identification of novel informative loci for DNA-based X-inactivation analysis, Blood Cells Mol. Dis. 54 (2015) 210–216.
[39] T. Tukiainen, A.C. Villani, A. Yen, M.A. Rivas, J.L. Marshall, R. Satija, M. Aguirre, L. Gauthier, M. Fleharty, A. Kirby, B.B. Cummings, S.E. Castel, K.J. Karczewski, F. Aguet, A. Byrnes, T. Lappalainen, A. Regev, K.G. Ardlie, N. Hacohen, D.G. Macarthur, Landscape of X chromosome inactivation across human tissues, Nature 550 (2017) 244–248.
[40] S. Niho, T. Yokose, K. Suzuki, T. Kodama, Y. Nishiwaki, K. Mukai, Monoclonality of atypical adenomatous hyperplasia of the lung, Am. J. Pathol. 154 (1999) 249–254.
[41] H. Li, Q. Chen, R. Zhu, D.H. Gu, H.G. Zhu, Clonality analysis of intraductal proliferative lesions using the human androgen receptor assay, Neoplasma 54 (2007) 490–494.
[42] L. Gong, L.X. Wei, P. Ren, W.D. Zhang, X.Y. Liu, X.J. Han, L. Yao, S.J. Zhu, M. Lan, Y.H. Li, W. Zhang, Dysplastic nodules with glypican-3 positive immunostaining: a risk for early hepatocellular carcinoma, PLoS One 9 (2014) e87120.
[43] Y. Ueda, T. Miyatake, M. Okazawa, T. Kimura, T. Miyake, K. Fujiwara, K. Yoshino, R. Nakashima, M. Fujita, T. Enomoto, Clonality and HPV infection analysis of concurrent glandular and squamous lesions and adenosquamous carcinomas of the uterine cervix, Am. J. Clin. Pathol. 130 (2008) 389–400.
[44] S. Zheng, O.W. Cummings, R. Saxena, S. Zhang, M. Wang, S.R. Williamson, M. Cheng, A. Lopez-Beltran, R. Montironi, K.B. Hodges, L. Cheng, Clonality and TP53 mutation analysis of focal nodular hyperplasia of the liver, Am. J. Clin. Pathol. 134 (2010) 65–70.
[45] Q. Yu, Y. Niu, Y. Yu, X. Ding, Y. Shi, Analysis of the progression of intraductal proliferative lesions in the breast by PCR-based clonal assay, Breast Cancer Res. Treat. 114 (2009) 433–440.
[46] L. Gong, Y.H. Li, Q. Su, G. Li, W.D. Zhang, W. Zhang, Use of X-chromosome inactivation pattern and laser microdissection to determine the clonal origin of focal nodular hyperplasia of the liver, Pathology 41 (2009) 348–355.
[47] A.M. Baker, T.A. Graham, N.A. Wright, Pre-tumour clones, periodic selection and clonal interference in the origin and progression of gastrointestinal cancer: potential for biomarker development, J. Pathol. 229 (2013) 502–514.

[48] M.R. Teixeira, S. Heim, Cytogenetic Analysis of Tumor Clonality, Adv. Cancer Res. (2011) 127–149.
[49] J.J. Salk, M.S. Horwitz, Passenger mutations as a marker of clonal cell lineages in emerging neoplasia, Semin. Cancer Biol. 20 (2010) 294–303.
[50] D.E. Brash, How do mutant clones expand in normal tissue? in: C.C. Maley, M. Greaves (Eds.), Frontiers in Cancer Research: Evolutionary Foundations, Revolutionary Directions, Springer New York, New York, NY, 2016pp. 61–98.
[51] N.E. Navin, The first five years of single-cell cancer genomics and beyond, Genome Res. 25 (2015) 1499–1507.
[52] A. Davis, N.E. Navin, Computing tumor trees from single cells, Genome Biol. 17 (2016) 113.
[53] M. Janiszewska, L. Liu, V. Almendro, Y. Kuang, C. Paweletz, R.A. Sakr, B. Weigelt, A.B. Hanker, S. Chandarlapaty, T.A. King, J.S. Reis-Filho, C.L. Arteaga, S.Y. Park, F. Michor, K. Polyak, In situ single-cell analysis identifies heterogeneity for PIK3CA mutation and HER2 amplification in HER2-positive breast cancer, Nat. Genet. 47 (2015) 1212–1219.
[54] N.E. Potter, L. Ermini, E. Papaemmanuil, G. Cazzaniga, G. Vijayaraghavan, I. Titley, A. Ford, P. Campbell, L. Kearney, M. Greaves, Single-cell mutational profiling and clonal phylogeny in cancer, Genome Res. 23 (2013) 2115–2125.
[55] Y. Wang, J. Waters, M.L. Leung, A. Unruh, W. Roh, X. Shi, K. Chen, P. Scheet, S. Vattathil, H. Liang, A. Multani, H. Zhang, R. Zhao, F. Michor, F. Meric-Bernstam, N.E. Navin, Clonal evolution in breast cancer revealed by single nucleus genome sequencing, Nature 512 (2014) 155–160.
[56] C. Yu, J. Yu, X. Yao, W.K. Wu, Y. Lu, S. Tang, X. Li, L. Bao, X. Li, Y. Hou, R. Wu, M. Jian, R. Chen, F. Zhang, L. Xu, F. Fan, J. He, Q. Liang, H. Wang, X. Hu, M. He, X. Zhang, H. Zheng, Q. Li, H. Wu, Y. Chen, X. Yang, S. Zhu, X. Xu, H. Yang, J. Wang, X. Zhang, J.J. Sung, Y. Li, J. Wang, Discovery of biclonal origin and a novel oncogene SLC12A5 in colon cancer by single-cell sequencing, Cell Res. 24 (2014) 701–712.
[57] J.M. Francis, C.Z. Zhang, C.L. Maire, J. Jung, V.E. Manzo, V.A. Adalsteinsson, H. Homer, S. Haidar, B. Blumenstiel, C.S. Pedamallu, A.H. Ligon, J.C. Love, M. Meyerson, K.L. Ligon, EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing, Cancer Discov. 4 (2014) 956–971.
[58] C. Gawad, W. Koh, S.R. Quake, Dissecting the clonal origins of childhood acute lymphoblastic leukemia by single-cell genomics, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 17947–17952.
[59] T. Baslan, J. Kendall, B. Ward, H. Cox, A. Leotta, L. Rodgers, M. Riggs, S. D'Italia, G. Sun, M. Yong, K. Miskimen, H. Gilmore, M. Saborowski, N. Dimitrova, A. Krasnitz, L. Harris, M. Wigler, J. Hicks, Optimizing sparse sequencing of single cells for highly multiplex copy number profiling, Genome Res. 125 (2015) 714–724.
[60] A.E.O. Hughes, V. Magrini, R. Demeter, C.A. Miller, R. Fulton, L.L. Fulton, W.C. Eades, K. Elliott, S. Heath, P. Westervelt, L. Ding, D.F. Conrad, B.S. White, J. Shao, D.C. Link, J.F. DiPersio, E.R. Mardis, R.K. Wilson, T.J. Ley, M.J. Walter, T.A. Graubert, Clonal architecture of secondary acute myeloid leukemia defined by single-cell sequencing, PLoS Genet. 10 (2014) e1004462.
[61] J. Kuipers, K. Jahn, N. Beerenwinkel, Advances in understanding tumour evolution through single-cell sequencing, Biochim. Biophys. Acta - Rev. Cancer 1867 (2017) 127–138.
[62] Y. Hou, L. Song, P. Zhu, B. Zhang, Y. Tao, X. Xu, F. Li, K. Wu, J. Liang, D. Shao, H. Wu, X. Ye, C. Ye, R. Wu, M. Jian, Y. Chen, W. Xie, R. Zhang, L. Chen, X. Liu, X. Yao, H. Zheng, C. Yu, Q. Li, Z. Gong, M. Mao, X. Yang, L. Yang, J. Li, W. Wang, Z. Lu, N. Gu, G. Laurie, L. Bolund, K. Kristiansen, J. Wang, H. Yang, Y. Li, X. Zhang, J. Wang, Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm, Cell 148 (2012) 873–885.
[63] M.B. Woodworth, K.M. Girskis, C.A. Walsh, Building a lineage from single cells: genetic techniques for cell lineage tracking, Nat. Rev. Genet. 18 (2017) 230–244.
[64] T.J. Phesse, V.M. Durban, O.J. Sansom, Defining key concepts of intestinal and epithelial cancer biology through the use of mouse models, Carcinogenesis 38 (2017) 953–965.
[65] A.T. Thliveris, L. Clipson, A. White, J. Waggoner, L. Plesh, B.L. Skinner, C.D. Zahm, R. Sullivan, W.F. Dove, M.A. Newton, R.B. Halberg, Clonal structure of carcinogen-induced intestinal tumors in mice, Cancer Prevent. Res. 4 (2011) 916–923.
[66] A.T. Thliveris, B. Schwefel, L. Clipson, L. Plesh, C.D. Zahm, A.A. Leystra, M.K. Washington, R. Sullivan, D.A. Deming, M.A. Newton, R.B. Halberg, Transformation of epithelial cells through recruitment leads to polyclonal intestinal tumors, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 11523–11528.
[67] S.I.J. Ellenbroek, J. Van Rheenen, Imaging hallmarks of cancer in living mice, Nat. Rev. Cancer 14 (2014) 406–418.
[68] C.D. Zahm, J.M. Szulczewski, A.A. Leystra, T.J.P. Olson, L. Clipson, D.M. Albrecht, M. Middlebrooks, A.T. Thliveris, K.A. Matkowskyj, M.K. Washington, M.A. Newton, K.W. Eliceiri, R.B. Halberg, Advanced intestinal cancers often maintain a multi-ancestral architecture, PLoS One 11 (2016) e0150170.
[69] A.A. Leystra, B. Luers, J. Son, C.K. Sievers, A.M. Wisinger, A.R. Schwartz, C.D. Zahm, K.A. Matkowskyj, D.M. Albrecht, L. Clipson, D.A. Deming, M.A. Newton, R.B. Halberg, Abstract 2381: a multiancestral model of colorectal cancer: &em&in vivo&/em& evidence that early heterogeneity contributes to cancer progression, Cancer Res. 76 (2016) 2381.
[70] A.G. Schepers, H.J. Snippert, D.E. Stange, M. Van Den Born, J.H. Van Es, M. Van De Wetering, H. Clevers, Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas, Science 337 (2012) 730–735.
[71] T. Tanaka, N. Atsumi, N. Nakamura, H. Yanai, Y. Komai, T. Omachi, K. Tanaka, K. Ishigaki, K. Saiga, H. Ohsugi, Y. Tokuyama, Y. Imahashi, H. Hisha, N. Yoshida, K. Kumano, K. Okazaki, H. Ueno, Bmi1-positive cells in the lingual epithelium could serve as cancer stem cells in tongue cancer, Sci. Rep. 6 (2016) 39386.
[72] H. Yanai, N. Atsumi, T. Tanaka, N. Nakamura, Y. Komai, T. Omachi, K. Tanaka, K. Ishigaki, K. Saiga, H. Ohsugi, Y. Tokuyama, Y. Imahashi, S. Ohe, H. Hisha, N. Yoshida, K. Kumano, M. Kon, H. Ueno, Intestinal cancer stem cells marked by Bmi1 or Lgr5 expression contribute to tumor propagation via clonal expansion, Sci. Rep. 7 (2017) 41838.
[73] M.Q. McCreery, A. Balmain, Clonal evolution of skin carcinomas during tumor progression and metastasis, Cancer Res. 76 (2016) 2376.
[74] R. Maddipati, B.Z. Stanger, Pancreatic cancer metastases harbor evidence of polyclonality, Cancer Discov. 5 (2015) 1086–1097.
[75] K.J. Lafaro, A. Hendley, J. Bailey, S. Leach, Clonal composition and clonal selection during PanIN progression, Cancer Res. 75 (2015) PR04.
[76] J.M. Fischer, A.G. Schepers, H. Clevers, D. Shibata, R.M. Liskay, Occult progression by Apc-deficient intestinal crypts as a target for chemoprevention, Carcinogenesis 35 (2014) 237–246.
[77] G.D. Dakubo, P. Danely, Slaughters field cancerization concept of carcinogenesis: the path forward to early cancer detection and chemoprevention, Cell Dev. Biol. 4 (2015) 158.
[78] P. Lochhead, A.T. Chan, R. Nishihara, C.S. Fuchs, A.H. Beck, E. Giovannucci, S. Ogino, Etiologic field effect: reappraisal of the field effect concept in cancer predisposition and progression, Mod. Pathol. 28 (2015) 14–29.
[79] T.A. Graham, S.A. McDonald, N.A. Wright, Field cancerization in the GI tract, Future Oncol. 7 (2011) 981–993.
[80] A. Marusyk, V. Almendro, K. Polyak, Intra-tumour heterogeneity: a looking glass for cancer? Nat. Rev. Cancer 12 (2012) 323–334.
[81] S.E. Woodman, G.B. Mills, Are oncogenes sufficient to cause human cancer? Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20599–20600.
[82] A. Davis, R. Gao, N. Navin, Tumor evolution: linear, branching, neutral or punctuated? Biochim. Biophys. Acta (BBA) - Rev. Cancer 1867 (2017) 151–161.
[83] B.L. Parsons, M.B. Myers, F. Meng, Y. Wang, P.B. McKinzie, Oncomutations as biomarkers of cancer risk, Environ. Mol. Mutagen. 51 (2010) 836–850.
[84] B.L. Parsons, K.L. McKim, M.B. Myers, Variation in organ-specific PIK3CA and KRAS mutant levels in normal human tissues correlates with mutation prevalence in corresponding carcinomas, Environ. Mol. Mutagen. 58 (2017) 466–476.
[85] H. Sudo, X.-C. Li-Sucholeiki, L.A. Marcelino, A.N. Gruhl, P. Herrero-Jimenez, H. Zarbl, J.C. Willey, E.E. Furth, S. Morgenthaler, H.A. Coller, P.O. Ekstrom, R. Kurzweil, E.V. Gostjeva, W.G. Thilly, Fetal–juvenile origins of point mutations in the adult human tracheal–bronchial epithelium: absence of detectable effects of age, gender or smoking status, Mutat. Res./Fundam. Mol. Mech. Mutagen. 646 (2008) 25–40.
[86] W. Gao, J. Jin, J. Yin, S. Land, A. Gaither-Davis, N. Christie, J.D. Luketich, J.M. Siegfried, P. Keohavong, KRAS and TP53 mutations in bronchoscopy samples from former lung cancer patients, Mol. Carcinog. 56 (2017) 381–388.
[87] F. Michor, K. Polyak, The origins and implications of intratumor heterogeneity, Cancer Prevent. Res. 3 (2010) 1361–1364.
[88] D. Neelakantan, D.J. Drasin, H.L. Ford, Intratumoral heterogeneity: clonal cooperation in epithelial-to-mesenchymal transition and metastasis, Cell Adhes. Migr. 9 (2015) 265–276.
[89] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, J. Clin. Invest. 119 (2009) 1420–1428.
[90] C.L. Chaffer, I. Brueckmann, C. Scheel, A.J. Kaestli, P.A. Wiggins, L.O. Rodrigues, M. Brooks, F. Reinhardt, Y. Suc, K. Polyak, L.M. Arendt, C. Kuperwasser, B. Bierie, R.A. Weinberg, Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 7950–7955.
[91] C. Köhler, D. Nittner, F. Rambow, E. Radaelli, F. Stanchi, N. Vandamme, A. Baggiolini, L. Sommer, G. Berx, J.J. van den Oord, H. Gerhardt, C. Blanpain, J.C. Marine, Mouse cutaneous melanoma induced by mutant BRaf arises from expansion and dedifferentiation of mature pigmented melanocytes, Cell Stem Cell 21 (2017) 679–693.
[92] T.A. Graham, N.A. Wright, Investigating the fixation and spread of mutations in the gastrointestinal epithelium, Future Oncol. 4 (2008) 825–839.
[93] D.P. Tabassum, K. Polyak, Tumorigenesis: it takes a village, Nat. Rev. Cancer 15 (2015) 473–483.
[94] S. Ramón y Cajal, C. Capdevila, J. Hernandez-Losa, L. de Mattos, A. Ghosh, J. Lorent, O. Larsson, T. Aasen, L.M. Postovit, I. Topisirovic, Cancer as an eco-molecular disease and a neoplastic consortium, Biochim. Biophys. Acta - Rev. Cancer 1868 (2017) 484–499.
[95] N. McGranahan, C. Swanton, Clonal heterogeneity and tumor evolution: past, present, and the future, Cell 168 (2017) 613–628.
[96] L. Ge, W. Meng, H. Zhou, N. Bhowmick, Could stroma contribute to field cancerization? Med. Hypotheses 75 (2010) 26–31.
[97] A.M. Soto, C. Sonnenschein, The tissue organization field theory of cancer: a testable replacement for the somatic mutation theory, BioEssays 33 (2011) 332–340.
[98] M. Wu, J.C. Pastor-Pareja, T. Xu, Interaction between RasV12 and scribbled clones induces tumour growth and invasion, Nature 463 (2010) 545–548.
[99] A.S. Cleary, T.L. Leonard, S.A. Gestl, E.J. Gunther, Tumour cell heterogeneitymaintained by cooperating subclones in Wnt-driven mammary cancers, Nature 509 (2014) 113–117.
[100] M.D.M. Inda, R. Bonavia, A. Mukasa, Y. Narita, D.W.Y. Sah, S. Vandenberg, C. Brennan, T.G. Johns, R. Bachoo, P. Hadwiger, P. Tan, R.A. DePinho, W. Cavenee, F. Furnari, Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma, Genes Dev. 24 (2010) 1731–1745.
[101] J.M. Bailey, A.M. Hendley, K.J. Lafaro, M.A. Pruski, N.C. Jones, J. Alsina, M. Younes, A. Maitra, F. McAllister, C.A. Iacobuzio-Donahue, S.D. Leach, P53 mutations cooperate with oncogenic Kras to promote adenocarcinoma from

pancreatic ductal cells, Oncogene 35 (2016) 4282–4288.
[102] M. Casás-Selves, J. DeGregori, How cancer shapes evolution, and how evolution shapes cancer, Evolution 4 (2011) 624–634.
[103] C. Hafner, A. Toll, A. Fernández-Casado, J. Earl, M. Marqués, F. Acquadro, M. Méndez-Pertuz, M. Urioste, N. Malats, J.E. Burns, M.A. Knowles, J.C. Cigudosa, A. Hartmann, T. Vogt, M. Landthaler, R.M. Pujol, F.X. Real, Multiple oncogenic mutations and clonal relationship in spatially distinct benign human epidermal tumors, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20780–20785.
[104] G.Y. Liou, H. Döppler, K.E. DelGiorno, L. Zhang, M. Leitges, H.C. Crawford, M.P. Murphy, P. Storz, Mutant KRas-Induced mitochondrial oxidative stress in acinar cells upregulates EGFR signaling to drive formation of pancreatic precancerous lesions, Cell Rep. 14 (2016) 2325–2336.
[105] C.K. Sievers, L.S. Zou, P.J. Pickhardt, K.A. Matkowskyj, D.M. Albrecht, L. Clipson, J.W. Bacher, B.D. Pooler, F.J. Moawad, B.D. Cash, M. Reichelderfer, T.N. Vo, M.A. Newton, B.R. Larget, R.B. Halberg, Subclonal diversity arises early even in small colorectal tumours and contributes to differential growth fates, Gut 66 (2016).
[106] C.K. Sievers, W.M. Grady, R.B. Halberg, P.J. Pickhardt, New insights into the earliest stages of colorectal tumorigenesis, Expert Rev. Gastroenterol. Hepatol. 11 (2017) 723–729.
[107] R. Fisher, L. Pusztai, C. Swanton, Cancer heterogeneity: implications for targeted therapeutics, Br. J. Cancer 108 (2013) 479–485.
[108] A.I. Rozhok, J. DeGregori, Toward an evolutionary model of cancer: considering the mechanisms that govern the fate of somatic mutations, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 8914–8921.
[109] S. Wu, S. Powers, W. Zhu, Y.A. Hannun, Substantial contribution of extrinsic risk factors to cancer development, Nature 529 (2016) 43–47.
[110] E.R. Fearon, B. Vogelstein, A genetic model for colorectal tumorigenesis, Cell 61 (1990) 759–767.
[111] A. Sottoriva, H. Kang, Z. Ma, T.A. Graham, M.P. Salomon, J. Zhao, P. Marjoram, K. Siegmund, M.F. Press, D. Shibata, C. Curtis, A Big Bang model of human colorectal tumor growth, Nat. Genet. 47 (2015) 209.
[112] W.C.H. Cross, T.A. Graham, N.A. Wright, New paradigms in clonal evolution: punctuated equilibrium in cancer, J. Pathol. 240 (2016) 126–136.
[113] C.K. Sievers, A.A. Leystra, L. Clipson, W.F. Dove, R.B. Halberg, Understanding intratumoral heterogeneity: lessons from the analysis of at-risk tissue and premalignant lesions in the colon, Cancer Prevent. Res. 9 (2016) 638.
[114] R. Gao, A. Davis, T.O. McDonald, E. Sei, X. Shi, Y. Wang, P.-C. Tsai, A. Casasent, J. Waters, H. Zhang, F. Meric-Bernstam, F. Michor, N.E. Navin, Punctuated copy number evolution and clonal stasis in triple-negative breast cancer, Nat. Genet. 48 (2016) 1119.
[115] R.A. Beckman, L.A. Loeb, Evolutionary dynamics and significance of multiple subclonal mutations in cancer, DNA Repair 56 (2017) 7–15.
[116] A.M. Nicholson, C. Olpe, A. Hoyle, A.-S. Thorsen, T. Rus, M. Colombe, R. Brunton-Sim, R. Kemp, P. Quirke, S. Malhotra, R. ten Hoopen, A. Ibrahim, M.B. Myers, B. Parsons, S. Tavare, M. Wilkinson, E. Morrissey, D.J. Winton, Fixation and spread of somatic clones in adult human colonic epithelium, Cell Stem Cell (2018) (in press).
[117] I.P.M. Tomlinson, Mutations in normal breast tissue and breast tumours, Breast Cancer Res. 3 (2001) 299.
[118] H. Huang, J. Daniluk, Y. Liu, J. Chu, Z. Li, B. Ji, C.D. Logsdon, Oncogenic K-Ras requires activation for enhanced activity, Oncogene 33 (2014) 532–535.
[119] I. Martincorena, P.J. Campbell, Somatic mutation in cancer and normal cells, Science 349 (2015) 1483–1489.
[120] C. Tomasetti, L. Li, B. Vogelstein, Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention, Science 355 (2017) 1330–1334.
[121] C. Tomasetti, B. Vogelstein, Variation in cancer risk among tissues can be explained by the number of stem cell divisions, Science 347 (2015) 78–81.
[122] W. Zhu, S. Wu, Y.A. Hannun, Contributions of the intrinsic mutation process to cancer mutation and risk burdens, EBioMedicine 24 (2017) 5–6.
[123] H. Sudo, X.C. Li-Sucholeiki, L.A. Marcelino, A.N. Gruhl, P. Herrero-Jimenez, H. Zarbl, J.C. Willey, E.E. Furth, S. Morgenthaler, H.A. Coller, P.O. Ekstrom, R. Kurzweil, E.V. Gostjeva, W.G. Thilly, Fetal-juvenile origins of point mutations in the adult human tracheal-bronchial epithelium: absence of detectable effects of age, gender or smoking status, Mutat. Res. - Fundam. Mol. Mech. Mutagen. 646 (2008) 25–40.
[124] E.J. Calabrese, Origin of the linearity no threshold (LNT) dose–response concept, Arch. Toxicol. 87 (2013) 1621–1633.
[125] L. Gong, X.L. He, Y.H. Li, K.X. Ren, L. Zhang, X.Y. Liu, X.J. Han, L. Yao, S.J. Zhu, M. Lan, W. Zhang, Clonal status and clinicopathological feature of Erdheim-Chester disease, Pathol. Res. Pract. 205 (2009) 601–607.
[126] K.M. Josten, J.A. Tooze, C. Borthwick-Clarke, E.C. Gordon-Smith, T.R. Rutherford, Acquired aplastic anemia and paroxysmal nocturnal hemoglobinuria: studies on clonality, Blood 78 (1991) 3162–3167.
[127] P.A. Beer, A.V. Jones, A.J. Bench, A. Goday-Fernandez, E.M. Boyd, K.J. Vaghela, W.N. Erber, B. Odeh, C. Wright, M.F. McMullin, J. Cullis, B.J.P. Huntly, C.N. Harrison, N.C.P. Cross, A.R. Green, Clonal diversity in the myeloproliferative neoplasms: independent origins of genetically distinct clones, Br. J. Haematol. 144 (2009) 904–908.
[128] A. Takatsu, T. Miyamoto, C. Fuseya, A. Suzuki, H. Kashima, A. Horiuchi, K. Ishii, T. Shiozawa, Clonality analysis suggests that STK11 gene mutations are involved in progression of lobular endocervical glandular hyperplasia (LEGH) to minimal deviation adenocarcinoma (MDA), Virchows Arch. 462 (2013) 645–651.
[129] M.Q. Almeida, L.C. Kaupert, L.P. Brito, A.M. Lerario, B.M.P. Mariani, M. Ribeiro, O. Monte, F.T. Denes, B.B. Mendonca, T.A.S.S. Bachega, Increased expression of ACTH (MC2R) and androgen (AR) receptors in giant bilateral myelolipomas from patients with congenital adrenal hyperplasia, BMC Endocr. Disord. 14 (2014) 42.
[130] C. Allen, J.R. Lambert, D.C. Linch, R.E. Gale, X chromosome inactivation analysis reveals a difference in the biology of ET patients with JAK2 and CALR mutations, Blood 124 (2014) 2091–2093.
[131] J.B.J. Kum, D.J. Grignon, M. Wang, M. Zhou, R. Montironi, S.S. Shen, S. Zhang, A. Lopez-Beltran, J.N. Eble, L. Cheng, Mixed epithelial and stromal tumors of the kidney: evidence for a single cell of origin with capacity for epithelial and stromal differentiation, Am. J. Surg. Pathol. 35 (2011) 1114–1122.
[132] X. Wang, M. Wang, G.T. MacLennan, F.W. Abdul-Karim, J.N. Eble, T.D. Jones, F. Olobatuyi, R. Eisenberg, O.W. Cummings, S. Zhang, A. Lopez-Beltran, R. Montironi, S. Zheng, H. Lin, D.D. Davidson, L. Cheng, Evidence for common clonal origin of multifocal lung cancers, J. Natl. Cancer Inst. 101 (2009) 560–570.
[133] W. Wang, J. Wang, S. Wang, D. Wang, J. Zhao, H. Zhu, Clonality assessment of adenomatoid tumor supports its neoplastic nature, Hum. Pathol. 48 (2016) 88–94.
[134] C.C. Gomes, C. Da Silveira Oliveira, W.H. Castro, J.C.T. De Lacerda, R.S. Gomez, Clonal nature of odontogenic tumours, J. Oral Pathol. Med. 38 (2009) 397–400.
[135] R.E. Emerson, M. Wang, L.M. Roth, W. Zheng, F.W. Abdul-Karim, F. Liu, T.M. Ulbright, J.N. Eble, L. Cheng, Molecular genetic evidence supporting the neoplastic nature of the Leydig cell component of ovarian Sertoli-Leydig cell tumors, Int. J. Gynecol. Pathol. 26 (2007) 368–374.
[136] A.D. Singhi, S. Norwood, T.C. Liu, R. Sharma, C.L. Wolfgang, R.D. Schulick, J.J. Zeh, R.H. Hruban, Acinar cell cystadenoma of the pancreas a benign neoplasm or non-neoplastic ballooning of acinar and ductal epithelium, Am. J. Surg. Pathol. 37 (2013) 1329–1335.
[137] S.L. Van Egmond, W.W.J. De Leng, F.H.M. Morsink, G.J.A. Offerhaus, L.A.A. Brosens, Monoclonal origin of primary unilateral multifocal pleomorphic adenoma of the parotid gland, Hum. Pathol. 44 (2013) 923–926.
[138] A. Chiu, M. Czader, L. Cheng, R.P. Hasserjian, M. Wang, S. Bhagavathi, E.M. Hyjek, H. Al-Ahmadie, D.M. Knowles, A. Orazi, Clonal X-chromosome inactivation suggests that splenic cord capillary hemangioma is a true neoplasm and not a subtype of splenic hamartoma, Mod. Pathol. 24 (2011) 108–116.
[139] F. Bourdeaut, A. Ribeiro, R. Paris, G. Pierron, J. Couturier, M. Peuchmaur, O. Delattre, In neuroblastic tumours, Schwann cells do not harbour the genetic alterations of neuroblasts but may nevertheless share the same clonal origin, Oncogene 27 (2008) 3066–3071.
[140] S.J. Holdsworth-Carson, M. Zaitseva, B.J. Vollenhoven, P.A.W. Rogers, Clonality of smooth muscle and fibroblast cell populations isolated from human fibroid and myometrial tissues, Mol. Hum. Reprod. 20 (2014) 250–259.
[141] M.T. Sung, M. Wang, G.T. MacLennan, J.N. Eble, P.H. Tan, A. Lopez-Beltran, R. Montironi, J.J. Harris, M. Kuhar, L. Cheng, Histogenesis of sarcomatoid urothelial carcinoma of the urinary bladder: evidence for a common clonal origin with divergent differentiation, J. Pathol. 211 (2007) 420–430.
[142] J.C. Sarubi, H. Bei, E.F. Adams, W.L. Boson, E. Friedman, K. Brandão, E. Kalapothakis, D. Miranda, F.L. Valle, M.S. Sarquis, L. De Marco, Clonal composition of human adamantinomatous craniopharyngiomas and somatic mutation analyses of the patched (PTCH), Gsα and Gi2α genes, Neurosci. Lett. 310 (2001) 5–8.
[143] L. Zhang, S. Comertpay, D. Shimizu, R.M. DeMay, M. Carbone, S.A. Honda, J.M. Matsuura Eaves, O.W. Tawfik, Axillary metaplastic breast carcinoma with ipsilateral pectoral invasive ductal carcinoma: an unusual presentation, Case Rep. Oncol. Med. 2014 (2014) 938509.
[144] K.B. Hodges, O.W. Cummings, R. Saxena, M. Wang, S. Zhang, A. Lopez-Beltran, R. Montironi, H. Nour, L. Cheng, Clonal origin of multifocal hepatocellular carcinoma, Cancer 116 (2010) 4078–4085.
[145] S. Comertpay, S. Pastorino, M. Tanji, R. Mezzapelle, O. Strianese, A. Napolitano, F. Baumann, T. Weigel, J. Friedberg, P. Sugarbaker, T. Krausz, E. Wang, A. Powers, G. Gaudino, S. Kanodia, H.I. Pass, B.L. Parsons, H. Yang, M. Carbone, Evaluation of clonal origin of malignant mesothelioma, J. Transl. Med. 12 (2014) 301.
[146] D. Yuan, W. XiuJuan, Z. Yan, L. JunQin, X. Fang, Y. Shirong, K. Xiaojing, F. Yanyan, W. Weidong, L. Dong, L. Qingli, Z. DeZhi, P. XiongMing, Use of X-Chromosome inactivation pattern to analyze the clonality of 14 female cases of Kaposi Sarcoma, Med. Sci. Monit. Basic Res. 21 (2015) 116–122.
[147] S. Bahrami, L. Cheng, M. Wang, T.D. Jones, J.C. Malone, S.D. Billings, Clonal relationships between epidermotropic metastatic melanomas and their primary lesions: a loss of heterozygosity and X-chromosome inactivation-based analysis, Mod. Pathol. 20 (2007) 821–827.
[148] T.M. Katona, T.D. Jones, M. Wang, J.N. Eble, S.D. Billings, L. Cheng, Genetically heterogeneous and clonally unrelated metastases may arise in patients with cutaneous melanoma, Am. J. Surg. Pathol. 31 (2007) 1029–1037.
[149] T. Nakazawa, T. Kondo, I. Tahara, K. Kasai, T. Inoue, N. Oishi, K. Mochizuki, T. Kubota, R. Katoh, Multicentric occurrence of multiple papillary thyroid carcinomas -HUMARA and BRAF mutation analysis, Cancer Med. 4 (2015) 1272–1280.
[150] L. Gong, K.X. Ren, Y.H. Li, X.Y. Liu, W.D. Zhang, L. Yao, S.J. Zhu, X.J. Han, L. Zhang, M. Lan, W. Zhang, Determination of clonal status of pulmonary sclerosing hemangioma with X-chromosome inactivation mosaicism and polymorphism of phosphoglycerate kinase and androgen receptor genes, Med. Oncol. 28 (2011) 913–918.
[151] Y. Wang, R.C. Wu, L.E. Shwartz, L. Haley, M.T. Lin, I.M. Shih, R.J. Kurman, Clonality analysis of combined Brenner and mucinous tumours of the ovary reveals their monoclonal origin, J. Pathol. 237 (2015) 146–151.
[152] F. Karino, T. Yokose, S. Matsukuma, Y. Miyagi, S. Murakami, H. Ito, H. Nakayama, K. Yamada, Clonality analysis performed using human androgen receptor assay in a rare case of undifferentiated thymic carcinoma coexisting with type AB thymoma, Pathol. Int. 66 (2016) 398–403.
[153] J. Lin, X. Song, C. Liu, Pelvic intravascular leiomyomatosis associated with benign pulmonary metastasizing leiomyoma: clinicopathologic, clonality, and copy

number variance analysis, Int. J. Gynecol. Pathol. 33 (2014) 140–145.
[154] Y.R. Cai, X.L. Diao, S.F. Wang, W. Zhang, H.T. Zhang, Q. Su, X-chromosomal inactivation analysis of uterine leiomyomas reveals a common clonal origin of different tumor nodules in some multiple leiomyomas, Int. J. Oncol. 31 (2007) 1379–1389.
[155] E. Tazaki, Y. Shishido-Hara, N. Mizutani, S. Nomura, H. Isaka, H. Ito, K. Imi, S. Imoto, H. Kamma, Histopathologcial and clonal study of combined lobular and ductal carcinoma of the breast, Pathol. Int. 63 (2013) 297–304.
[156] L. Gong, Y.H. Li, J. Wang, X.Y. Liu, W. Zhang, c-KIT gene mutation and clonal origin of multiple gastrointestinal stromal tumors occurring in the peritoneum, Mol. Med. Rep. 2 (2009) 999–1004.
[157] L. Cheng, G.T. Mac Lennan, S. Zhang, M. Wang, M. Zhou, P.H. Tan, S. Foster, A. Lopez-Beltran, R. Montironi, Evidence for polyclonal origin of multifocal clear cell renal cell carcinoma, Clin. Cancer Res. 14 (2008) 8087–8093.
[158] M.T. Sung, S. Zhang, G.T. MacLennan, A. Lopez-Beltran, R. Montironi, M. Wang, P.H. Tan, L. Cheng, Histogenesis of clear cell adenocarcinoma in the urinary tract: evidence of urothelial origin, Clin. Cancer Res. 14 (2008) 1947–1955.
[159] S.R. Williamson, P.J. Bunde, R. Montironi, A. Lopez-Beltran, S. Zhang, M. Wang, G.T. MacLennan, L. Cheng, Malignant perivascular epithelioid cell neoplasm (PEComa) of the urinary bladder with TFE3 gene rearrangement clinicopathologic, immunohistochemical, and molecular features, Am. J. Surg. Pathol. 37 (2013) 1619–1626.
[160] T.A. Graham, S. Khan, A. Green, D. Oukrif, L. Gay, H. Barr, M. Novelli, J. Jankowski, N.A. Shepherd, S.J. Leedham, S.A.C. McDonald, N.A. Wright, Investigating clonal competition in barrett's associated tumorigenesis using spatial maps of genetic heterogeneity, Gut 59 (2010) A111.
[161] C. Thirlwell, O.C.C. Will, E. Domingo, T.A. Graham, S.A.C. McDonald, D. Oukrif, R. Jeffrey, M. Gorman, M. Rodriguez-Justo, J. Chin-Aleong, S.K. Clark, M.R. Novelli, J.A. Jankowski, N.A. Wright, I.P.M. Tomlinson, S.J. Leedham, Clonality assessment and clonal ordering of individual neoplastic crypts shows polyclonality of colorectal adenomas, Gastroenterology 138 (2010) 1441–1454.
[162] N. Navin, A. Krasnitz, L. Rodgers, K. Cook, J. Meth, J. Kendall, M. Riggs, Y. Eberling, J. Troge, V. Grubor, D. Levy, P. Lundin, S. Maner, A. Zetterberg, J. Hicks, M. Wigler, Inferring tumor progression from genomic heterogeneity, Genome Res. 20 (2010) 68–80.
[163] L. Gutierrezgonzalez, T.A. Graham, M. Rodriguezjusto, S.J. Leedham, M.R. Novelli, L.J. Gay, T. Ventayol-Garcia, A. Green, I. Mitchell, D.L. Stoker, S.L. Preston, S. Bamba, E. Yamada, Y. Kishi, R. Harrison, J.A. Jankowski, N.A. Wright, S.A.C. McDonald, The clonal origins of dysplasia from intestinal metaplasia in the human stomach, Gastroenterology 140 (2011) 1251–1260.
[164] T. Ventayol Garcia, M. Rodriguez-Justo, T. Graham, M. Novelli, N. Wright, S. McDonald, The clonal origins of gastric adenocarcinoma, Gut 61 (2012) A43–A44.
[165] E. Waanders, B. Scheijen, L.T. van der Meer, S.V. van Reijmersdal, L. van Emst, Y. Kroeze, E. Sonneveld, P.M. Hoogerbrugge, A.G. van Kessel, F.N. van Leeuwen, R.P. Kuiper, The origin and nature of tightly clustered BTG1 deletions in precursor B-cell acute lymphoblastic leukemia support a model of multiclonal evolution, PLoS Genet. 8 (2012) e1002533.
[166] M.J. Walter, D. Shen, L. Ding, J. Shao, D.C. Koboldt, K. Chen, D.E. Larson, M.D. McLellan, D. Dooling, R. Abbott, R. Fulton, V. Magrini, H. Schmidt, J. Kalicki-Veizer, M. O'Laughlin, X. Fan, M. Grillot, S. Witowski, S. Heath, J.L. Frater, W. Eades, M. Tomasson, P. Westervelt, J.F. Dipersio, D.C. Link, E.R. Mardis, T.J. Ley, R.K. Wilson, T.A. Graubert, Clonal architecture of secondary acute myeloid leukemia, New Engl. J. Med. 366 (2012) 1090–1098.
[167] S.S. Zeki, S. Khan, T.A. Graham, S. Leedham, H. Barr, N.A. Shepherd, M. Novelli, M. Rodriguez-Justo, N.A. Wright, S.A. McDonald, Barretts related adenocarcinomas arise as a monoclonal outgrowth from polyclonal dysplasia, Gastroenterology 142 (2012) S643–S644.
[168] J.M.J. Weaver, C.S. Ross-Innes, N. Shannon, A.G. Lynch, T. Forshew, M. Barbera, M. Murtaza, C.A.J. Ong, P. Lao-Sirieix, M.J. Dunning, L. Smith, M.L. Smith, C.L. Anderson, B. Carvalho, M. O'Donovan, T.J. Underwood, A.P. May, N. Grehan, R. Hardwick, J. Davies, A. Oloumi, S. Aparicio, C. Caldas, M.D. Eldridge, P.A.W. Edwards, N. Rosenfeld, S. Tavaré, R.C. Fitzgerald, S.J. Hayes, A. Yeng, A.M. Lydon, S. Dharmaprasad, S. Greer, S. Preston, S. Oakes, V. Save, S. Paterson-Brown, O. Tucker, D. Alderson, P. Taniere, J. Kelly, J. Byrne, D. Sharland, N. Holling, L. Boulter, F. Noble, B. Stacey, C. Crichton, H. Barr, N. Shepherd, L.M. Almond, O. Old, J. Lagergren, J. Gossage, A. Davies, R. Mason, F. Chang, J. Zylstra, G. Sanders, T. Wheatley, R. Berrisford, T. Bracey, C. Harden, D. Bunting, T. Roques, J. Nobes, S. Loo, M. Lewis, E. Cheong, O. Priest, S.L. Parsons, I. Soomro, P. Kaye, J. Saunders, V. Pang, N.T. Welch, J.A. Catton, J.P. Duffy, K. Ragunath, L. Lovat, R. Haidry, H. Miah, S. Kerr, V. Eneh, R. Butawan, L. Igali, H. Ford, D. Gilligan, P. Safranek, A. Hindmarsh, V. Sudjendran, A. Metz, N. Carroll, M. Scott, A. Cluroe, A. Miremadi, B. Mahler-Araujo, O. Knight, B. Nutzinger, C. Peters, Z. Abdullahi, I. Debriram-Beecham, S. Malhotra, J. Crawte, S. MacRae, A. Noorani, R.F. Elliott, X. Li, L. Bower, A. Achilleos, J. Bornschein, S. Zeki, H. Chettouh, M. Secrier, N. De Silva, E. Gregson, T.P. Yang, J.R. O'Neil, Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis, Nat. Genet. 46 (2014) 837–843.
[169] B. Li, J.Z. Li, A general framework for analyzing tumor subclonality using SNP array and DNA sequencing data, Genome Biol. 15 (2014) 473.
[170] Y.J. Gu, Y.Y. Zhu, X.Y. Lu, Q. Zhao, W.M. Cong, Hepatic carcinosarcoma: evidence of polyclonal origin based on microsatellite analysis, Pathol. Res. Pract. 211 (2015) 905–910.
[171] P.C. Boutros, M. Fraser, N.J. Harding, R. De Borja, D. Trudel, E. Lalonde, A. Meng, P.H. Hennings-Yeomans, A. McPherson, V.Y. Sabelnykova, A. Zia, N.S. Fox, J. Livingstone, Y.J. Shiah, J. Wang, T.A. Beck, C.L. Have, T. Chong, M. Sam, J. Johns, L. Timms, N. Buchner, A. Wong, J.D. Watson, T.T. Simmons, C. P'ng, G. Zafarana, F. Nguyen, X. Luo, K.C. Chu, S.D. Prokopec, J. Sykes, A.D. Pra, A. Berlin, A. Brown, M.A. Chan-Seng-Yue, F. Yousif, R.E. Denroche, L.C. Chong, G.M. Chen, E. Jung, C. Fung, M.H.W. Starmans, H. Chen, S.K. Govind, J. Hawley, A. D'Costa, M. Pintilie, D. Waggott, F. Hach, P. Lambin, L.B. Muthuswamy, C. Cooper, R. Eeles, D. Neal, B. Tetu, C. Sahinalp, L.D. Stein, N. Fleshner, S.P. Shah, C.C. Collins, T.J. Hudson, J.D. McPherson, T. Van Der Kwast, R.G. Bristow, Spatial genomic heterogeneity within localized, multifocal prostate cancer, Nat. Genet. 47 (2015) 736–745.
[172] C.C. Gomes, C.F. Galvão, A.C. do Carmo, N.B. Pereira, R.S. Gomez, Intratumor molecular heterogeneity in pleomorphic adenoma of the salivary glands, Oral. Surg. Oral Med. Oral Pathol. Oral Radiol. 121 (2016) 158–163.
[173] M. Cereda, G. Gambardella, L. Benedetti, F. Iannelli, D. Patel, G. Basso, R.F. Guerra, T.P. Mourikis, I. Puccio, S. Sinha, L. Laghi, J. Spencer, M. Rodriguez-Justo, F.D. Ciccarelli, Patients with genetically heterogeneous synchronous colorectal cancer carry rare damaging germline mutations in immune-related genes, Nat. Commun. 7 (2016), http://dx.doi.org/10.1038/ncomms12072.
[174] M. Gausachs, E. Borras, K. Chang, S. Gonzalez, D. Azuara, A.D. Amador, A. Lopez-Doriga, F.A.S. Lucas, X. Sanjuan, M.J. Paules, M.W. Taggart, G.E. Davies, E.A. Ehli, J. Fowler, V. Moreno, M. Pineda, Y.N. You, P.M. Lynch, C. Lazaro, N.E. Navin, P.A. Scheet, E.T. Hawk, G. Capella, E. Vilar, Mutational heterogeneity in APC and KRAS arises at the crypt level and leads to polyclonality in early colorectal tumorigenesis, Clin. Cancer Res. 23 (2017) 5936–5947.
[175] A.M. Baker, M. Wang, X.J. Ma, C. Anderson, W. Huang, E. Domingo, A. Lewis, J. Bridgewater, M. Jansen, N.A. Wright, M. Rodriguez-Justo, E. Park, I. Tomlinson, T.A. Graham, Visualization of treatment resistant subclones in colorectal cancer by mutation specific RNA in situ hybridization, Cancer Res. 77 (2017) 3953.
[176] N. Navin, J. Kendall, J. Troge, P. Andrews, L. Rodgers, J. McIndoo, K. Cook, A. Stepansky, D. Levy, D. Esposito, L. Muthuswamy, A. Krasnitz, W.R. McCombie, J. Hicks, M. Wigler, Tumour evolution inferred by single-cell sequencing, Nature 472 (2011) 90–95.
[177] X. Xu, Y. Hou, X. Yin, L. Bao, A. Tang, L. Song, F. Li, S. Tsang, K. Wu, H. Wu, W. He, L. Zeng, M. Xing, R. Wu, H. Jiang, X. Liu, D. Cao, G. Guo, X. Hu, Y. Gui, Z. Li, W. Xie, X. Sun, M. Shi, Z. Cai, B. Wang, M. Zhong, J. Li, Z. Lu, N. Gu, X. Zhang, L. Goodman, L. Bolund, J. Wang, H. Yang, K. Kristiansen, M. Dean, Y. Li, J. Wang, Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor, Cell 148 (2012) 886–895.
[178] Y. Li, X. Xu, L. Song, Y. Hou, Z. Li, S. Tsang, F. Li, K.M. Im, K. Wu, H. Wu, X. Ye, G. Li, L. Wang, B. Zhang, J. Liang, W. Xie, R. Wu, H. Jiang, X. Liu, C. Yu, H. Zheng, M. Jian, L. Nie, L. Wan, M. Shi, X. Sun, A. Tang, G. Guo, Y. Gui, Z. Cai, J. Li, W. Wang, Z. Lu, X. Zhang, L. Bolund, K. Kristiansen, J. Wang, H. Yang, M. Dean, J. Wang, Single-cell sequencing analysis characterizes common and cell-lineage-specific mutations in a muscle-invasive bladder cancer, GigaScience 1 (2012) 12.
[179] A.P. Patel, I. Tirosh, J.J. Trombetta, A.K. Shalek, S.M. Gillespie, H. Wakimoto, D.P. Cahill, B.V. Nahed, W.T. Curry, R.L. Martuza, D.N. Louis, O. Rozenblatt-Rosen, M.L. Suvà, A. Regev, B.E. Bernstein, Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma, Science 344 (2014) 1396–1401.
[180] A.G. Knudson, Mutation and human cancer, Adv. Cancer Res. 17 (1973) 317–352.
[181] P. Nowell, The clonal evolution of tumor cell populations. Acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression, Science 194 (1976) 23–28.
[182] E. Fearon, S.R. Hamilton, B. Vogelstein, Clonal analysis of human colorectal tumors, Science 238 (1987) 193–196.
[183] B. Vogelstein, E. Fearon, S.R. Hamilton, A.P. Feinberg, Use of restriction fragment length polymorphisms to determine the clonal origin of human tumors, Science 227 (1985) 642–645.
[184] M.F. Lyon, Gene action in the x-chromosome of the mouse (Mus Musculus L.), Nature 190 (1961) 372–373.
[185] D. Linder, S.M. Gartler, Glucose-6-phosphate dehydrogenase mosaicism: utilization as a cell marker in the study of leiomyomas, Science 150 (1965) 67–69.
[186] P.J. Fialkow, R.W. Sagebiel, S.M. Gartler, D.L. Rimoin, Multiple cell origin of hereditary neurofibromas, N. Engl. J. Med. 284 (1971) 296–300.