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Chapter 2

Morphological Markers of Chromosomal Instability

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Abstract

Cancer is characterized by genomic complexity and chromosomal instability (CIN). Atypical mitosis and nuclear atypia such as micronuclei have been reported as morphological characteristics of chromosomal instability. An atypical mitotic figure is defined as anything other than the typical form of normal mitosis, including multipolar, ring, dispersed, asymmetrical, and lag-type mitoses. A micronucleus is defined as the small nucleus that forms whenever a chromosome or its fragment is not incorporated into one of the daughter nuclei during cell division. A telomere plays a key role in chromosomal instability. Telomere dysfunction induces fusion of chromatids and chromosome missegregation and this phenomenon can be observed as abnormal mitotic figures and micronuclei. Detection of morphological markers of chromosomal instability using pathological specimens, even small biopsy or cytological specimens, may provide valuable information concerning the prognosis of cancers. Here, we discuss morphological assessment of chromosomal instability using routine pathological specimens.

Keywords: chromosomal instability, cancer, pathology, mitosis, atypical mitosis

1. Introduction

Cancer is characterized by genomic complexity and chromosomal instability (CIN); mutations of cancer-related genes, telomere dysfunction, aneuploidy, polyploidy, nuclear atypia, and abnormal mitosis are all contributors to this phenotype [1–4]. The greatest risk factor for cancer is considered to be aging, via telomere shortening, accumulation of mutations, and perturbations in the microenvironment [5, 6]. Previously, we showed that age-related shortening of telomere length in various tissues is correlated to aging-related diseases, such as cancers,

diabetes mellitus, and cognitive disorders [7]. Telomere shortening often occurs in cancers, as well as in precancerous lesions [8–10]. Telomere shortening induces fusion of chromatids and chromosome missegregation and this phenomenon can be observed as abnormal mitotic figures and micronuclei. In this article, we discuss the morphological markers to determine chromosomal instability in cancer tissues.

2. Chromosomal instability

CIN is defined as a persistently high rate of loss or gain of full or partial chromosomes induced by defects in cohesion, the spindle assembly checkpoint, centrosomes, kinetochore-microtubule attachment dynamics, or cell cycle regulation [11, 12]. Cells with CIN make errors in chromosome segregation in approximately 20% of cell divisions and the unequal distribution of DNA to daughter cells upon mitosis induces a failure to maintain euploidy leading to aneuploidy. Most solid tumors and hematological cancers are aneuploidy and many missegregate chromosomes at very high rates [11]. However, the presence of aneuploidy in cells does not necessarily mean CIN is present; a high rate of errors is definitive of CIN. Detection of CIN requires the determination of chromosome missegregation rates, however the ability to detect CIN from fixed tumor tissues is limited [13]. Therefore, when we need to determine CIN using fixed tumor samples in the clinical setting, we usually perform indirect methods such as karyotype analysis, fluorescent *in situ* hybridization, or array-based comparative genomic hybridization analyses. Analysis of atypical mitotic figures and nuclear atypia is considered a useful method to distinguish chromosomally unstable from chromosomally stable malignancies [14–18].

3. Mitotic figures

Mitosis is divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase. During mitosis, chromosomes thicken and condense, allowing them to be visualized by light microscopy. Most malignant tumors show a high mitotic index and for some tumors, a diagnosis of malignancy is based on mitotic index. A higher mitotic index is correlated with malignancy grade and prognosis [19, 20]. Structural chromosomal abnormalities may arise during somatic cell divisions. Cells with CIN have a higher probability of causing chromosome missegregation during mitosis as compared to normal cells, suggesting a close relationship between high mitotic index and CIN in malignancies, possibly as a result of mitotic arrest as opposed to high frequency of mitoses.

Cytological smears and formalin-fixed paraffin-embedded tissue samples are useful materials for evaluating mitotic figures because they are routinely performed in laboratories around the world. Metaphase figures can be evaluated using hematoxylin and eosin (H&E), Giemsa, or Papanicolaou-stained slides examined at high power magnification. The mitotic index value is assessed by counting the number of mitoses per 1000 or 2000 nuclei or per 50 high power fields.

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Mitotic figures are defined as figures without a nuclear membrane, which indicates that the cell has passed prophase and in which clear hairy extensions of nuclear material are present. Pyknotic nuclei or nuclei with basophilic cytoplasm are not thought to distinguish mitosis from apoptosis or degenerative cells [21]. Recently, immunohistochemical determination of proliferating cells using primary antibodies for Ki67, PCNA, or phosphohistone H3 has become popular; however, sometimes there is a discrepancy between mitotic index and Ki67 index [22–24]. We believe that this phenomenon represents the frequent mitotic arrest mentioned above.

4. Atypical mitosis

Mitosis is classified into normal and atypical mitosis [25]. An atypical mitotic figure is defined as anything other than the typical form of normal mitosis, including an anaphase bridge, multipolar, ring, dispersed, asymmetrical, and lag-type mitoses [25, 26] (**Figure 1**). Cells in mitosis are often seen in normal tissues exhibiting rapid turnover, such as the epithelium of the gut, but the most important morphologic features of malignancy are atypical and bizarre mitotic figures. In our analysis, 30% of mitosis in pancreatic cancer cells was atypical mitosis, while normal epithelium did not show atypical mitosis and precancerous lesions showed only a few instances of atypical mitosis [9, 18].

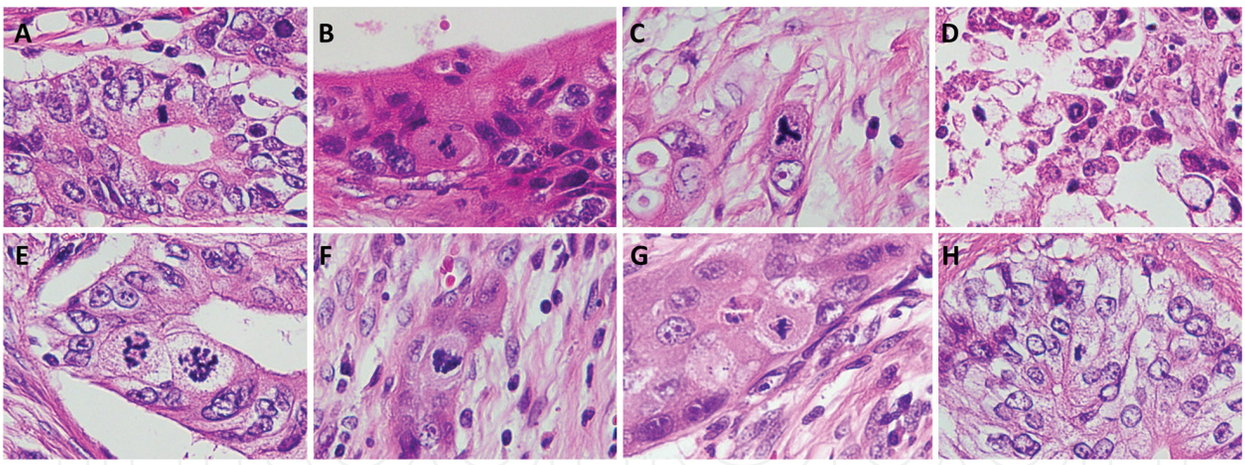

Figure 1. Normal and atypical mitosis in cancer cells. A, normal mitosis; B, anaphase bridge; C, multipolar mitosis; D, ring mitosis; E, dispersed mitosis; F, asymmetrical mitosis; G, lag-type mitosis; and H, micronuclei. H&E stain. Original magnification 400×.

An anaphase bridge is defined as a filamentous connection linking two well-separated and parallel-aligned groups of anaphase chromosomes [14, 15]. Telomeres protect each end of the chromosome from fusion; therefore, telomere dysfunction can be observed as an anaphase bridge [15, 17]. A lot of evidence has shown that telomere dysfunction plays a key role in carcinogenesis via induction of CIN [9, 27]; thus, detection of an anaphase bridge has been considered a useful method of indirectly evaluating telomere dysfunction and CIN.

Multipolar mitosis is metaphase with an abnormal configuration of the equatorial plate and the chromosomes are located along several radial axes. These figures are subdivided into tripolar mitoses, quadripolar mitoses, and others. Multipolar mitosis might be associated with multipolar spindles and numerical and functional abnormalities of centrosomes [28, 29]. It has been reported that multipolar mitosis determined by cytologic smears is useful to distinguish malignancies from benign tissue [30, 31]. Recently, we have reported that the existence of multipolar mitosis, but not other atypical mitotic figures, was an independent prognostic factor for in pancreatic cancers [18]. Multipolar mitosis-positive pancreatic cancer cases may have high invasiveness into surrounding tissue and arteries, in part, because of chromosomal instability and abnormality of the centrosome.

Lag-type mitoses are figures with nonattached condensed chromatin in the area of the mitotic figure. These are subdivided into metaphases with nonattached condensed chromatin at one polar side, metaphases with nonattached condensed chromatin at equidistant positions at the two polar sides and others. Furuta et al. has reported lag-type mitosis as a marker of high-risk human papilloma virus associated cervical cancers [32].

Medication-induced atypical mitoses have been reported. Docetaxel, paclitaxel, and colchicine can cause mitotic arrest, ring mitoses, and epithelial atypia mimicking dysplasia [33, 34]. They bind to the β-tubulin subunit of the microtubules of the mitotic spindle apparatus and therefore prevent mitotic spindle formation.

The interrelationship of each atypical mitotic figure has not been well clarified; however, each type of atypical mitosis is a morphologically important marker of CIN.

5. Telomere dysfunction

Aging drives telomere dysfunction. Inflammation, alcohol drinking, and diabetes mellitus also accelerate telomere attrition [35–37]. Furthermore, telomere shortening initiates the early phase of carcinogenesis even when there are no histopathological changes [9, 17]. Telomere dysfunction can be seen as nuclear atypia including the presence of micronuclei, nuclear buds, and anaphase bridges [38]. In our analysis, telomere length in the normal pancreatic duct was negatively correlated with mitotic index [9], which is consistent with telomere shortening of 100 base pairs in each mitosis. Normal epithelial cells in pancreatic cancer patients showed shorter telomeres than those in patients without cancers. Furthermore, telomere shortening was correlated to *KRAS* mutation in pancreatic cancer. These data indicate that telomere shortening occurs prior to CIN and drives CIN [39]. As a result, CIN drives gene mutation, deletion, or amplification.

In addition to this pathway, microsatellite instability also induces genetic abnormality and there seems to be organ specificity. Some colon and uterine cancers are caused by microsatellite instability [40], but most pancreatic cancers are microsatellite stable. All of the conventional pancreatic ductal adenocarcinomas showed telomere dysfunction and it progressed

according to malignancy grade of pancreatic carcinogenesis steps [9]. Organ specificity as well as the difference of carcinogens might influence such difference of carcinogenesis steps. In CIN cancers, mitosis and atypical mitosis might have a predictive value of malignancy grade and prognosis [18].

6. Morphological markers of chromosomal instability

The usefulness of micronuclei in distinguishing malignant lesions from benign lesions using cytological specimens has been well clarified [41–43]. A micronucleus is the small nucleus that forms whenever a chromosome or its fragment is not incorporated into one of the daughter nuclei during cell division and it serves as an indicator of CIN. Samanta et al. reported that in the evaluation of the number of micronuclei in 1000 cells from fine needle aspiration samples of the breast, cancer cells showed a higher number of micronuclei than benign lesions [44]. Tyagi et al. assessed the number of anaphase bridges, multipolar mitoses, micronuclei, and nuclear budding in 1000 cells in Giemsa stained smears of ascitic fluid and found that these markers were correlated with the cytological diagnosis [30]. Moreover, Verma and Dey counted anaphase bridges, multipolar mitoses per smear, micronuclei and nuclear budding per 1000 carcinoma cells using fine needle aspiration samples of breast cancer and these markers were correlated with cytological grades [31]. We also counted normal and atypical mitoses in 1000 cells using surgically resected pancreatic cancer tissues and they were correlated with tumor stage and prognosis [18]. The number of mitotic figures is sometimes very low even in cancer tissues. For example, the mitotic index of pancreatic cancers was only 0.4%, suggesting the potential need to analyze more than 1000 cells [45].

Micronuclei, nuclear budding, anaphase bridging, and multipolar mitoses have been well evaluated among various morphological markers of CIN. The molecular methods to determine CIN are costly, require expertise, and may not be available in many laboratories. In the future, these aforementioned markers can be applied to diagnose malignancy in difficult cases of suspected malignancy.

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