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Volume 676

Polyploidization and Cancer

Edited by
Randy Y.C. Poon

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DEDICATION

For my parents

PREFACE

Limiting genome replication to once per cell cycle is vital for maintaining genome stability. Although polyploidization is of physiological importance for several specialized cell types, inappropriate polyploidization is believed to promote aneuploidy and transformation. A growing body of evidence indicates that the surveillance mechanisms that prevent polyploidization are frequently perturbed in cancers.

Progress in the past several years has unraveled some of the underlying principles that maintain genome stability. This book brings together leaders of the field to overview subjects relating to polyploidization and cancer. The importance of polyploidization in the evolution of cancer is discussed by Merlo, Wang, Pepper, Rabinovitch, and Maley. Proper execution of mitosis is controlled by the spindle-assembly checkpoint and is paramount in preventing mitotic slippage and polyploidization. Ito and Matsumoto discuss our current understanding of this checkpoint. Cytokinesis failure is another important route to polyploidization. A discourse on the mechanisms that lead to cytokinesis failure and their relationship to genome instability is provided by Normand and King. The evidence of a role of DNA damage in polyploidization is also discussed (Chow and Poon). In normal cells, polyploidization is prevented by p53-dependent mechanisms. Salient features of these pathways are described by Talos and Moll. As discussed by Duensing and Duensing, defective mitosis caused by supernumerary centrosomes is increasingly being recognized for their roles in causing polyploidy and cancer. Furthermore, important examples of polyploidization including hematopoietic cells (Nguyen and Ravid) and liver cells (Celton-Morizur and Desdouets) serve to illustrate the pivotal role of polyploidization in cancers and senescence. Last but not least, state-of-the-art methodologies of how ploidy can be measured are detailed by Darzynkiewicz, Halicka, and Zhao.

I thank the various authors for their invaluable contribution. Much remains to be learned about the regulation of mitosis, cytokinesis, centrosome duplication, checkpoints, and their relationship to polyploidization and tumorigenesis. It is hoped that these articles will serve as a resource for further progress of this important area of cancer research.

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CONTENTS

Chapter 1

Polyploidy, Aneuploidy and the Evolution of Cancer

Lauren M. F. Merlo, Li-san Wang, John W. Pepper, Peter S. Rabinovitch and Carlo C. Maley*

Abstract

Aneuploidy is a ubiquitous feature of cancer and pre-cancerous lesions, yet its significance is poorly characterized. In this chapter, we review the role of tetraploidy and aneuploidy in progression. We examine how aneuploidy may contribute to the evolutionary dynamics prevalent in neoplastic progression, considering whether aneuploidy itself is selectively neutral or advantageous or if it simply acts as a mechanism for the more rapid accumulation of mutations increasing survival and reproduction of cancer cells. We also review evidence from Barrett's esophagus, a pre-malignant condition, demonstrating that tetraploidy and aneuploidy are correlated with an increased risk of progression to cancer. Ultimately, we aim provide testable hypotheses and methods for understanding the role of aneuploidy in cancer.

Introduction

Most cancers cells are aneuploid, meaning they contain the wrong number of chromosomes. Aneuploidy entails the loss or gain of individual chromosomes or large sections of chromosomes and is defined here as distinct from polyploidy, which involves extra copies of the entire genome, such as triploidy (3N) or tetraploidy (4N). Large-scale chromosomal amplifications and deletions in cancers have been demonstrated using a variety of methods, including comparative genomic hybridization (CGH), karyotyping and fluorescence in situ hybridization (FISH).[1-3]

The frequency with which aneuploidy is observed in cancer leads to a series of important questions: How do cancers become aneuploid? What genes are being targeted by the amplifications and deletions? How can cells survive with such massive perturbations to their genomes? How has the selective pressure of cancer shaped our genomes to sense and respond to these perturbations? Most of these questions have received little attention to date and the answers remain largely unknown. To facilitate research on these questions, we review what is known about each in an effort to frame the questions and hypotheses more precisely and propose methods that might be used to reach an answer.

The Tetraploidy to Aneuploidy Progression in Carcinogenesis

Abnormalities in chromosome content were observed to be common in tumor cells at least 120 years ago.[4] The hypothesis that genomic instability could result from whole genome doublings (tetraploidy) and that this could play an important role in cancer was made more than a century ago by Theodor Boveri.[5] For many years these observations were neglected, as aneuploidy and

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tetraploidy were commonly held to be incidental to tumor evolution. However, within the past several decades the importance of chromosomal abnormalities and chromosomal instability has risen. When aneuploid DNA content became readily identifiable by flow cytometry, it was noted that this finding was more common in higher grade cancers and that aneuploid tumors of many kinds had a more aggressive clinical behavior than their diploid counterparts.[6,7] Today, some authors argue that the evidence points to aneuploidy playing a pivotal role in the chromosomal instability that generates tumor diversity, clonal evolution and malignant phenotypes.[8]

While aneuploidy could in principal be generated by progressive additions to the diploid DNA content by accumulated chromosomal gains, as by mitotic nondisjunction, it would then be puzzling that aneuploid tumor DNA contents are most commonly in the triploid to tetraploid range.[9] Boveri's hypothesis allows that a tetraploid intermediate is a common precursor to aneuploidy and that subsequent chromosomal evolution by loss of superfluous chromosomes or chromosome segments results in the aneuploid chromosomal complement. A conceptual model of the role of the tetraploid intermediate in carcinogenesis was formalized by Shackney et al.[10] Supporting experimental evidence comes from observations of a tetraploid intermediate during murine carcinogenesis.[11,12] Furthermore, when diploid and tetraploid mouse cells from a common mammary precursor were directly compared, the tetraploid cells had greater chromosomal instability and only the tetraploid cells gave rise to malignant tumors when transplanted into nude mice.[13] Perhaps most significantly, tetraploidy has been demonstrated to be a precursor of aneuploidy in several human cancers, including Barrett's esophagus (see below) and cervical carcinoma.[14] The mechanisms that underlie generation of the tetraploid state are now recognized to include the failure of cytokinesis and, in particular, failure of checkpoint control during mitosis.[15] Loss of p53 function plays an important role in augmenting this process, as failure of p53-dependent G1 checkpoint and DNA repair commonly result in G2/M checkpoint arrest; failure of this latter checkpoint, or accommodation or "slippage," allows cells to reenter the cell cycle with a failure of cytokinesis, resulting in tetraploid G1 cells.[15,16]

Tetraploidy and Aneuploidy in Barrett's Esophagus

It is difficult to determine the role of polyploidy and aneuploidy in the development of cancer because most cancers cannot be studied longitudinally. When we detect a neoplasm we either remove it or, if it has metastasized, treat it systemically (which may generate additional aneuploid cells). The same is true for most premalignant neoplasms. This prevents us from studying the effects of ploidy changes on the further development of the neoplasm and from making direct observations of the ordering of events in progression. An important exception is Barrett's esophagus (BE).

Barrett's esophagus is a premalignant neoplasm[17] that predisposes for the development of esophageal adenocarcinoma (EA).[18] Characterized by the presence of specialized intestinal epithelium in the esophagus, it can be recognized endoscopically as a salmon-colored epithelium just above the gastro-esophageal sphincter. Only about 0.5% of people with BE progress to EA per year and most people with BE will die of some other cause.[19] Unlike other premalignant neoplasms, such as an adenomatous polyp in the colon, BE is not removed when detected. Esophagectomies have an 8%-23% mortality rate[20] and thus the risk of progression to EA does not justify the risk of removal of the BE segment. Instead, the standard of care is surveillance with periodic endoscopic biopsies for the early detection of cancer. If EA is detected in an intensive surveillance program, it is often caught prior to metastasis and patients can be treated surgically. For these purely clinical reasons, BE presents a scientific opportunity to study the genetics of how a neoplasm changes over time as it progresses to cancer.

We study BE as a model of neoplastic progression in solid tumors. Aside from the danger of removing it and ease of biopsying it, BE is similar to many other conditions that predispose to carcinogenesis in a variety of respects. Like inflammatory bowel disease, hepatitis, pancreatitis, prostatitis, *H. pylori* infection in the stomach and *Schistosomiasis* infection in the bladder, BE is characterized by chronic inflammation.[21] Similar to other premalignant conditions, only a minority of patients with BE progress to cancer. In addition, neoplastic progression in BE is characterized by some of the most common genetic lesions across all cancers: loss of the tumor suppressor genes p16 (INK4A/CDKN2A) and

p53 (TP53) and the development of tetraploidy and aneuploidy. Studying BE provides us the major advantage of observing the development of these lesions over time. What have these longitudinal studies taught us about the role of polyploidy and aneuploidy in neoplastic progression?

p16

The first genetic and epigenetic lesion commonly observed in BE is loss of the p16 tumor suppressor gene. Tlsty and colleagues have argued that loss of p16 leads to decoupling of the synthesis of DNA and centrosomes in the cell cycle, such that if either is delayed, the cell might enter mitosis with the wrong number of centrosomes or the wrong amount of DNA and aneuploidy could result.[22] It is unclear if this happens in BE. To date, the association between the loss of p16 and ploidy abnormalities has not been adequately studied. We do know that patients can live for many years lacking p16 in their BE neoplasm but never develop aneuploidy.

p53

The genetic lesion that has been associated with the development of both tetraploidy and aneuploidy in BE is loss of the p53 tumor suppressor.[23,24] Our current hypothesis is that although aneuploid cells may arise in a p53 wildtype clone, they normally trigger the p53-dependent DNA damage checkpoint which either leads to senescence or apoptosis and so the aneuploid clone never grows large enough to be sampled. Once the p53 checkpoint is compromised, aneuploid clones are free to proliferate without check. This is why we believe that the loss of p53 precedes the development of both tetraploidy and aneuploidy. Loss of heterozygosity at the p53 locus is also the strongest single predictor of progression and is associated with a 16-fold increased risk of progression to EA[24] as well as a 6-fold increased risk of developing tetraploidy and a 7.5-fold increased risk of developing aneuploidy.

Tetraploidy

Tetraploidy, defined in this case as greater than 6% of cells with 4N DNA content, is also a predictor of progression associated with a 12-fold (95% CI: 6.2-22) increased risk of progression to EA.[25] Sometimes this may be an indication of cells being stalled in the G2 phase of the cell cycle. Other times, the presence of 8N cells in cell cycle analysis suggests that there are viable tetraploid cells in the neoplasm. FISH studies have found that loss of heterozygosity in p53 as detected by microsatellite analysis could be caused by deletion of one allele of p53 or, more often, by duplication of the genome followed by deletion of multiple p arms of chromosome 17 where p53 resides.[26]

Aneuploidy

Most cases of aneuploid clones in BE have DNA content between diploidy and tetraploidy further suggesting that tetraploidy is an intermediate stage of progression followed by selective loss of parts of the genome. This appears to be true of other cancers as well.[9,27-29] We have compiled a survey of 57 esophageal adenomas that were surgically removed prior to therapy and analyzed for DNA content in our study (Fig. 1). This new data agrees with our previously published data[25] that hypodiploids and supratetraploids are rare.

The detection of an aneuploid clone in BE is associated with a 9.5-fold (95% CI: 4.9-18) increased risk of progression.[25] However, the presence of both tetraploidy and aneuploidy is an indication of greater risk of progression than either alone[25,30] and may be a sign of more extensive genomic instability.

It should be noted that in BE, at least, the loss of p53 and the development of aneuploidy is not sufficient to cause cancer. In contrast, loss of p53 is thought to cause malignancy in colorectal carcinogenesis.[31] Although BE patients with both a p53 lesion and a ploidy lesion (either tetraploidy or aneuploidy) are at a very high risk of progressing to cancer, that process can still take years.[30] So there must be other loci that are being targeted by the gains and losses during the further evolution of aneuploid clones. Hopefully, genome-wide analyses of aneuploid BE and EA will reveal the final genetic lesions that cause invasion and metastasis.