HORMONAL CARCINOGENESIS IV
Hormonal Carcinogenesis IV

Proceedings of the
Fourth International Symposium
HORMONAL CARCINOGENESIS IV

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In Memoriam to

Charles Bretton Huggins, M.D.

Ben May Laboratories
for Cancer Research
University of Chicago
Nobel Laureate, 1960

This volume is dedicated to the following individuals who unstintingly lent their resources and/or facilities to these Symposia since its inception in 1991, thus contributing importantly to our collective understanding of hormonal carcinogenesis and hormonal cancer research.

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Preface

It has been over a decade since the First International Symposium on Hormonal Carcinogenesis convened in 1991. Since then, the field has rapidly expanded with considerable progress in both breast and prostate cancers; while ovarian and endometrial cancer have been hampered, in part, due to the absence of suitable hormone-mediated animal models. While knock-out, transgenic, and cell-culture systems have been extremely useful in identifying specific gene/protein alterations and the ensuing pathways affected, the precise molecular mechanisms whereby sex hormones elicit their oncogenic effects still remain elusive. Moreover, despite the considerable progress made in breast cancer research, the exact role of progestins in the presence or absence of estrogen in breast growth, differentiation, and malignant transformation is lacking. Elucidating the incipient molecular alterations in early/pre-invasive lesions elicited by these hormones is a growing important focus of this field.

The main purpose of these Symposia has been to address vital questions that impact our understanding of the causation, dependency, progression, resistance, and prevention of hormonally-associated cancers.

We are indebted to the Scientific Advisory Board members who worked with us reviewing and offering suggestions to finalize the scientific program. We offer special thanks for the guidance and support of Dr. Gerald Mueller. His wisdom played an indispensable role in maintaining the excellence of these Symposia. We also acknowledge the numerous external reviewers that worked diligently to revise and improve the quality of the manuscripts. We are very grateful to Ms. Tandria Price. Her enthusiasm for the project, her effective and diplomatic interactions with contributors and administrators, and her superb organizational skills were evident during the Symposium and in the preparation of this volume. We are deeply grateful to the Fundación Instituto Valenciano Oncología (IVO), Universidad de Valencia, and the Office of Science and Culture of the Valencia Community, who hosted this Symposium and provided funds and gracious staff that worked efficiently to make this a most memorable Symposium. We are indebted to Ms. Paula Callaghan, our Springer-Verlag editor, for her support and highest publication standards. We appreciate the financial support of the NIH institutes and companies, listed separately, which have been indispensable to the success of this Symposium.

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Symposium Address

Hormones, Centrosomes, and Genomic Instability in Mammary Carcinogenesis

William R. Brinkley, David L. Stenoien, and Thea Goepfert

Introduction

The centrosome, a cytoplasmic organelle acquired at fertilization via paternal inheritance, plays a vital role in cell division and in the maintenance of cell polarity throughout development. The importance of the centrosome in achieving proper orientation and segregation of duplicated chromosomes and assuring stability of the eukaryotic genome is well established. The centrosome can be recognized in the cytoplasm of most eukaryotic cells as a discrete, microscopic domain that functions as the cell’s principal microtubule organizing center (MTOC). Like the genome, it undergoes duplication during late S-phase, producing a pair of centrosomes that function to organize the bipolar spindle in mitosis.

The centrosome duplication cycle, like the cell cycle, is regulated by a vital system of checkpoint-signaling proteins, about which little is currently known. Errors in centrosome duplication and/or distribution can result in aberrant daughter cells that either lack centrosomes (acentric cells) or receive a single centrosome, producing mono-polar spindles, or they can receive more than two centrosomes, resulting in the assembly of multipolar spindles during mitosis. These aberrations can lead to catastrophic errors in chromosome distribution resulting either in cell death or transformation to become tumor cells. Using antibodies specific for centrosome associated proteins, we and others have noted that many tumors, both in-vitro and in-vivo, display cells with a variety of centrosome aberrations (1-7). The most commonly reported defect causes cells to develop supernumerary centrosomes (greater than the expected 1-2 centrosomes/cell), a process identified as “centrosome amplification.” Retention and maintenance of centrosome duplicity is critical in the cell cycle to assure bipolar spindles and the maintenance of a diploid genome. Centrosome amplification could therefore account, in part, for the genomic instability and aneuploidy commonly found in cancer cells. Although bipolar spindles can form in the absence of centrosomes in some germ cells of eukaryotes this organelle plays a prominent role in spindle assembly and function in somatic cells (reviewed in 5). Thus, it becomes essential to identify and characterize the molecular components involved in maintaining the correct
centrosome number in diploid cells and to understand the cause of aberrations.

Although little is known about the role of hormones in the biology of centrosomes, they serve an important role in normal cell growth and developments, and are implicated in tumorigenesis. In a later section of this report, we review recent studies in our laboratory of centrosomes and their involvement in hormone mediated aneuploidy and cancer in an experimental rat mammary model for carcinogenesis.

The Resurrection

The implication of centrosome anomalies in cancer were first identified at the dawn of the 20th Century. In a 1914 treatise, *Zur Frage der Entstehung Maligner Tumoren*, Theodore Boveri (8) proposed that sea urchin zygotes containing more than two centrosomes, segregated their chromosomes abnormally due to the presence of extra spindle poles induced by polyspermy (Figure 1). He noted that although multipolarity was generally lethal, occasional segregants survived to produce embryos with tumor-like outgrowths. As one of the early experts on the role of centrosomes in cell division, Boveri was the first to propose a direct link between oncogenesis and the presence of multipolar spindles, aneuploidy and loss of tissue architecture in these embryos. Although he never studied cancer cells per se, his astute observations of this simple invertebrate system allowed him to derive many important postulates that still apply to cancer, including the concept of oncogenes, cell cycle checkpoints, tumor-suppressor genes, genetic instability, the clonal origin of tumors, chromosome specific “weakness” (telomeres), loss of cell adhesion, and genetic mosaicism. Moreover, he achieved this monumental task with little more than a primitive light microscope, a keen sense of observation and a truly remarkable intuition.

Boveri’s novel hypothesis that centrosome anomalies could be responsible for aneuploidy and the ontogeny of cancer, created a brief but spirited debate at the time, but was never widely accepted by the cancer establishment and it was essentially ignored until recently. The development and rise of *Drosophila* genetics by the Morgan school of genetics (9) and the discovery by Muller, et al. (10) that x-rays were potent mutagens and carcinogens, led to the widely accepted view that cancer is caused by a somatic gene mutation. The subsequent discovery of cellular oncogenes and tumor suppressor genes in the latter half of the 20th Century added considerable reinforcement to the somatic mutation idea (11-12). Despite the complex genetic basis of cancer, mutations in somatic genes remains the accepted hypothesis for oncogenesis by most cancer researchers today (for alternative view, see 13). However, recent reports of centrosome anomalies associated with many tumor cells (3-6), along with the well-established role of this organelle in mitotic spindle assembly and chromosome segregation (6, 7, 14), has revived Boveri’s 96 year-old hypothesis that aberrant centrosomes and aneuploidy (genomic instability) are incipient events in oncogenesis.
The role of centrosomes in cancer resurfaced again in 1996 when Fukasawa, et al. (1) reported centrosome amplification in mouse embryonic fibroblast null for the tumor suppressor p53. This group used anti-γ tubulin antibodies to detect and count centrosomes by immunofluorescence and reported that cells with the p53 null phenotype displayed more than the normal complement of centrosomes, whereas wild type and heterozygous cells displayed normal numbers of centrosomes. Three additional manuscripts published in 1998 from the laboratories of Roop and Brinkley (2), Salisbury (3), Pihan, Doxy, et al. (4) reported centrosome anomalies, especially centrosome amplification, in tumor cells in vivo. These reports were followed by additional findings that centrosomal abnormalities are common to many human cancers (reviewed in 6) and have sparked a lively resurrection of Boveri’s original hypothesis (14).

The Enlightenment: The Aurora Family of Serine/Threonine Kinases

The remarkable re-discovery of centrosome amplification in many common cancers led to a renewed interest in aneuploidy in neoplasia and initiated a search for a cellular and/or biochemical mechanism responsible for this phenomenon. It was immediately obvious; however that progress would be hampered by a dearth of knowledge about the molecular basis of centrosome maturation and replication in
eukaryotic cells (15, 16). Theoretically, centrosome amplification (more than the normal 1-2 centrosomes/cell) can occur by one of several pathways: (a) through a defect in a checkpoint pathway that controls centrosome duplication in late S-phase resulting in the over duplication, (b) via failure to partition duplicated centrosomes into daughter cells due to arrested or aberrant cytoplasmic cleavage of cytokinesis (6, 17), or (c) due, possibly, to fragmentation of pericentriolar material into small dispersed bodies that retain their capacity serve as MTOCs.

New light was cast onto the mechanism of centrosome amplification with the discovery of mitotic serine/threonine kinases representing the Aurora kinase family that included the prototypic yeast *Ipl1* and the *Drosophila Aurora* kinases (reviewed in 18, 19). Two groups from the laboratories of Sen and Brinkley at Houston (20) and the Bischoff group at Los Angeles (21) reported elevated expression of Aurora A (AurA) in many human cancers. Moreover, antibodies made against AurA were found to localize to the centrosomes of both interphase and mitotic cells (Figure 2).

**Figure 2.** Localization and expression of Aurora kinase in mammalian cells. (A) Antibodies against AurA are localized in the centrosomes of HeLa cells and when the gene, STK15 is overexpressed by transfection, multiple centrosomes appear. Two centrosomes appear in cells transfected with the vector, while 20% of the STK15 transfected cells displayed > 3 centrosomes/cell. (B) Centrosomes counts in 200 vector and STK15-transfected cells are shown in (C) Figures 1D-E show growth of cells in agar of stable transfected cells with vector (D) and NIH 3T3 cell transfected with STK 15 and grown in 0.5% bovine calf serum. Micrographs were taken at a total magnification of X100. F, Western blot analysis of STK 15-transfected 3T3 clones showing expression of STK15. [From Figure 5 in Zhou, et al., 1998 (20)].