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INVITED PERSPECTIVE

Boveri at 100: Theodor Boveri and genetic predisposition to cancer#

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#This article is part of a short series that was invited in relation to a Symposium held at the European Congress of Pathology, London, September 2014, that celebrated the centenary of the publication, in 1914, of Theodor Boveri's seminal work *Zur Frage der Entstehung maligner Tumouren*.

Abstract

One hundred years have passed since the publication of Theodore Boveri's *Zur Frage der Entstehung maligner Tumouren* [Concerning the Origin of Malignant Tumours]. This prescient publication created the foundations for much of our understanding of the origins of cancer and in particular the genetic basis of some cancers. In his work, Boveri suggested that loss of key cellular attributes, now known as tumour suppressor genes, are a key driver event in the development of cancer and inheritance could play a role in cancer susceptibility. He also predicted that chromosomal (genomic) instability as a key hallmark of cancer. Whilst these key insights that still inform the practice of cancer genetics, they were not the main theme of Boveri's text, which was to describe the role of chromosomal abnormalities in the development of cancer. In making his case he also suggested that genetic information could be contained in distinct packages (genes) that are linearly arranged along chromosomes and that cancers arise from single cells. These remarkably accurate hypotheses add weight to the need to celebrate this landmark publication for its accurate prediction of so much that we take for granted. Here we focus on Boveri's contributions to our understanding of hereditary cancers, which, alongside the astute clinical observations of Paul Broca and Aldred Scott Warthin, were published decades before the field became respectable, yet could still inform anyone studying hereditary cancers.

Keywords: cancer; hereditary; genetics; chromosomal theory; chromosome; tumour suppressor gene; genomic instability; inheritance

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Introduction

Our understanding of the relationship between genetics and cancer, and how we could use this clinically, dates back well beyond the sequencing of the human genome or the bloom of cancer genetics that occurred in the era of gene cloning in the 1990s, or even well before the work of Knudson or Henry Lynch. The roots of this field were planted by astute clinicians such as Paul Broca, who described the first hereditary breast cancer family, and Aldred Scott Warthin, who described a family with Lynch syndrome, and the imaginative basic scientist, Theodor Boveri [1–3]. Boveri used experimental evidence empowered by early cytogenetics techniques, along with an understanding of Mendel's rediscovered contributions [4] to develop the concepts that underpin much that we understand today. When one considers that this contribution was from a rank outsider, who used the eggs of sea urchins (Echinoidea) to study the basis of inheritance, Boveri's 'Concerning the Origin of Malignant Tumours' [3] seems even more remarkable. From his observation that abnormal inherited chromosomal arrangement or numbers impact the development of sea urchins, in combination with a broad-ranging understanding of his era's biological and cancer research, he derived multiple insights. Some, such as the suggestion that 'heritable transmission can only exist in the sense that a particular predisposition is transmitted' are so elegantly stated that improving upon them when describing these principles to students may be impossible [3].

This early hypothesis of viewing the tumour problem as a cellular rather than an organ problem or field defect laid the foundation for future biologists to confirm that cancer is, no doubt, a genetic disease. Boveri provided experimental evidence that Mendel's hereditary factors were chromosomes [3]. Independently of Boveri's work, Walter Sutton worked on his hypothesis of reduction division from maternal and paternal inheritance of chromosomes (later entitled 'meiosis') [5]. He suggested that this process provided the physical evidence

for Mendel's Law of Heredity [5]. These synergistic, independent insights have been jointly described as the Boveri–Sutton chromosome theory. However, Boveri's view that tumours arise from improper inheritance of genetic material (chromosomes), laid the groundwork for today's understanding of cancer predisposition. Boveri's primal and instinctive conclusions, that there is 'a sharp distinction between malignant tumours that arise from a hereditary component, and those that do not', which can be seen in the age of onset and other clinical features of hereditary cancers, highlight how significant his contributions truly were to the field [3].

In his early work, Boveri focused his efforts on the organization of chromosomes in sea urchins during cellular division [3,6]. Working with other cell biologists, such as Richard Hertwig, Boveri published a series of papers on the mechanism of cell division, meiosis and fertilization of sea urchin eggs [6–8]. One early paper in particular focused on the role of centrosomes during cell division [7], which unknowingly played a critical role in his later works on chromosomes and their contribution to inheritance and malignancy. Boveri provided the first experimental evidence that sperm and egg cells each contribute an equal number of chromosomes to a zygote [6,7]. Using this information, he began to address questions regarding the consistency of this number and hypothesized that deviations through abnormal cell division would lead to atypical growth patterns in cell progeny. He surmised that if a nuclear defect and abnormal chromosome inheritance was harmless, then all chromosomes would be of the same value [3]. This led to the groundbreaking hypothesis that individual chromosomes have qualitative differences and that the correct inheritance of all such properties is required for life [3], a statement that was initially met with scepticism but was later confirmed.

The key observations and, retrospectively speaking, contributions made to the study of hereditary cancers are outlined in his last work, 'Concerning the Origin of Malignant Tumours' [3]. It is here that Boveri foreshadows the early hypothesis of tumour suppressor genes, genomic instability and others that collectively contribute to today's widely accepted understanding of the hallmarks of cancer and inherited predisposition.

Early idea of tumour suppressor genes

Boveri's early theory, that the proliferative cells of malignant tumours are clearly missing something that is present in normal cells, undoubtedly foreshadowed today's understanding of tumour suppressor genes. Boveri postulated that, among the differentiating characteristics of chromosomes, there exist 'inhibitory chromosomes' inherited in normal cells that act to control cell growth and unrestrained multiplication under normal conditions [3], a fascinating prediction made at a time when genes were not yet described. He hypothesized that it is not until an external stimulus overcomes this mechanism that cell division can take place. These 'inhibitory chromosomes' are physically removed in malignant cells, allowing them to proliferate without constraint [3]. Boveri also assumed that the loss of such chromosomes in malignant cells is the cause of an irreparable inherit defect. Of note, Boveri cites the simultaneous studies of Hertwig and collaborators, who showed that irradiation acts on the nucleus and that malignant tumour cells 'succumb more easily to these rays than the nucleus of a normal cell' [9]. Translated into today's jargon, these observations refer to environmental mutagenesis.

Prior to Boveri's experimental evidence to support the essential inheritance of chromosomes and their contribution to cancer, astute clinicians had described the first well-annotated families with overt cancer susceptibility. Paul Broca, in 1866, wrote of his own family and the many incidents of breast cancer [1]. He predicted that the predisposition to tumours can be specific to certain tissues and that recurrence of these tumours is an 'inevitable fate' in some families [1]. Broca hypothesized that the reasoning behind delayed onset of cancer could be that the cause of such diseases remains dormant or inactive from birth in an undefined state [1], which would later be described as 'disease predisposition'. Although Broca makes very clear that the inheritance of cancer is a rare occurrence and only a theory at the time, it is clear that his theories were accurate and were used as groundwork for future discoveries.

In 1913, Aldred Scott Warthin, an American pathologist, also known for inventing the Warthin–Starry stain, published an article entitled 'Heredity with reference to carcinoma' [2]. Warthin carried forward Broca's theories by examining pathological specimens and genealogy charts from a single family ('family G'), in which multiple members succumbed to, or were treated for, cancers [2]. He, too, concluded that a remarkable pattern of cancer exists in certain family generations, which could only be ascertained as a predisposition to disease [2]. Anecdotally, the family he described was that of his seamstress; today, Warthin would be remarkable as both a pathologist with deep curiosity and insight as well as being able to afford that degree of household help! Decades later, 'family G' was followed up by a team led by Dr Henry Lynch, who documented many cases of colon, rectum, stomach and endometrial cancers and characterized it as a Lynch syndrome family [10]; the family was subsequently found to have a heritable mutation in the mismatch repair pathway (*MLH1* gene) [11].

The observations of Broca and Warthin represent the first known studies of organ-specific predispositions to cancer. Along with the experimental evidence and insights of Boveri, they can be considered the foundation of cancer genetics.

The field bloomed in the 1970s with the discovery of the first tumour suppressor gene, *Rb* or Retinoblastoma, and comprehensive understanding of Knudson's two-hit hypothesis, which states that for a tumour

suppressor gene to be inactivated and ultimately lead to malignancy, both copies must be lost [12, 13]. This inactivation comes from the inheritance of only one functional copy (ie heterozygosity at that specific locus) and subsequent loss of the second allele later in life through environmental factors or somatic mutation. The inherited loss of one functional allele is what aids to the predisposition to the disease, as the likelihood of losing both copies to genetic abnormality and developing a disorder is much greater – as was predicted by Boveri. Although, Knudsen did not cite Boveri in the classic articles cited above he did recognize Boveri's contribution to the concept of tumour suppressor genes in a review article written 25 years later [14]. Likewise there is no direct evidence that the other clinicians and researchers who described the major cancer susceptibility syndromes in the 1960's and 70's knew of his predictions [15, 16].

With today's cutting-edge technologies, such as relatively cheap testing of panels of potential cancer susceptibility genes, finding mutations is easy; yet interpreting their clinical and biological relevance is still challenging. In the interpretation of results from such testing, the principles presented by Boveri and other founders of the hereditary cancer field remain of use.

Cancer derives from a single cell and diffuses: genomic instability and clonal expansion

In conjunction with early understanding of the existence of tumour suppressor genes were Boveri's early observations of genomic instability and clonal expansion in cancers. One of the most significant topics in the aforementioned publication [3] was the idea that, no matter how dispersed a tumour may be, all of its cells are derived from cells present when the tumour is first formed. In other words, Boveri stated that every tumour originates from a single cell origin, a concept later translated into today's understanding of clonal expansion. He further elaborated that it is the faulty inheritance and abnormal assembly of chromosomes that initiates the tumourigenic effect in the primordial cell [3]. Boveri concludes that inheriting such an imperfect assembly of chromosomes gives rise to uncontrolled proliferation and mitotic events and, consequently, such aberrations will be passed onto that cells progeny and malignancy will form [3]. It is only by inheriting the proper combination of individual chromosomes, and their differentiating characteristics, that a cell can function normally and stay alive [3]. He used this hypothesis to support the idea that tumours to not arise as diffuse entities but grow from a single origin, unnoticed by the host. To elaborate this, Boveri provided support that tumours often arise in places of chronic irritation. He stated that chronic irritation has the power to produce chromosomal abnormalities during mitosis, while providing the necessary environmental conditions to which this small number of tumour cells can divide and proliferate [3]. Today, the associated concepts of intratumoural heterogeneity and the clonal evolution of cancers are two of the most intensely studied topics in cancer research.

Boveri described the fundamental role of chromosomal instability in the development of cancers. We now know that chromosomal instability, including the accumulation of extra copies, large deletions and chromosomal translocations, is but one of many ways in which cancer genomes become permissive to new mutations. It is likely that Boveri would have been impressed to see that so many of the genes responsible for hereditary cancers, including *BRCA1* and *BRCA2*, are classified as tumour suppressor genes directly involved in the maintenance of chromosomal integrity through the repair of double-stranded breaks in chromosomes [17]. Many other cancer susceptibility genes, such as genes associated with Lynch syndrome (*MLH1*, *MSH2* and *MSH6*), impact genomic integrity albeit operating on subtler mutations [18, 19]. Boveri also described cell death as a response to inheriting an abnormal complement of chromosomes [3]. The avoidance of such a fate in cancer cells is often acquired through *TP53* mutation, which is both the most common mutation event in cancer and, when inherited in the germline, the cause of Li–Fraumeni syndrome [20].

Boveri made other early hypotheses using chromosomal theory that have translated into different themes under the hereditary cancer umbrella. For example, he postulated that: '… if an impairment of particular chromosomes can occur in such a way that … they do not split properly during karyokinesis, both homologues in the diploid chromosome set would have to be impaired in the same way for a tumour to be produced' [3]. Boveri elaborates this testimony by assuming that two of the homologues would need the same defect in both parental gametes, reaching the conclusion that inbreeding increasing the rate of occurrence or tumours [3]. He refers to the disease xeroderma pigmentosum, which we now know today as an autosomal recessive disorder, as an example of the heritability of predisposition, and stated that this disease occurred most frequently in the progeny of close relatives [3]. This is an impressive early description of autosomal recessive inheritance and further emphasizes Boveri's intuitive observations of molecular genetics. Eighty years after these observations, it was shown the xeroderma pigmentosa is caused by the inherited defects that impact genomic stability [21].

Along with autosomal recessive diseases and genetic linkage, Boveri commented on an even more complex phenomenon, germline mosaicism. During fertilization, some gametes may contain a mutation while the others are normal, resulting in only a certain percentage of the germline cells of the body containing such a mutation and increasing the risk for disease in those cells alone. Boveri describes this event as aberrant chromosomal distribution throughout the body, and used it as support for explaining why multiple occurrences of the same tumour may be embedded at various sites in an

otherwise healthy organism [3]. He hypothesized that asymmetrical mitosis may occur sooner than later during development, and result in some individual daughter cells neglecting to receive the necessary assembly of chromosomes [3]. As support for this argument, Boveri mentions specific species that can exist entirely as one sex. He claims that in these hermaphrodite organisms, the entire body of an individual is a complex of male and female regions through asymmetrical mitosis in some cells of the embryo [3]. He draws the conclusion that similar events may occur in the bodies of healthy individuals that possess a small percentage of cells with a predisposition to malignancy [3]. This remarkable observation was not confirmed experimentally until the 1930s, where it was shown that genetic recombination could, on occurrence, take place during early mitosis of fetal development [22]. Today, with advanced sequencing technologies, there is an improved understanding of genetic mosaic mutations and their contribution to disease. Sex-linked syndromes, such as Klinefelter syndrome, whereby certain cells of an affected male contain an extra X chromosome, as well as somatic, postzygotic acquisition of activating *PI3KCA* mutations in patients with CLOVES syndrome [23], are several examples of how certain cells mosaic with respect to genetics of the tissues. The potential role of mosaicism in cancer is an area of active investigation. As this field emerges, there will likely be an increasing appreciation of Boveri's insights.

It is difficult to say whether Boveri's thoughts on tumour suppressor genes and the potential inheritance of cancer directly influenced the field of cancer genetics or if his predictions can be viewed as astoundingly accurate but too advanced to be absorbed and further developed by the scientific and medical communities at the time. Unfortunately, the fifty-year gap between his observations and serious consideration of the impact of genetics on cancer risk may have led to a diminution of appreciation for his work by those who study hereditary cancer. In reviewing seminal papers that profoundly impacted our understanding of cancer susceptibility (Joseph Fraumeni, Henry Lynch, Alfred Knudson), there is no mention of Boveri or his early predictions [12, 13, 15, 16]. It is possible that these pioneers of modern cancer genetics were not entirely aware of Boveri's ideas pertaining to the inheritance of cancer predisposition, rather their curiosity and imagination led them conclusions that could be seamlessly later spliced to these lost threads of ideas from Boveri.

Theodor Boveri is justifiably considered to be one of the most important biologists of his generation and the one hundredth anniversary of the publication of his "Concerning the Origin of Malignant Tumours" presents a great opportunity to celebrate his insights.

Author contributions

Both authors contributed to the research, writing and editing of this review.

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