Cancer: Cell Structures, Carcinogens and Genomic Instability
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Cancer morphology, carcinogenesis and genetic instability: a background

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Summary. Morphological abnormalities of both the nuclei and the cell bodies of tumour cells were described by Müller in the late 1830s. Abnormalities of mitoses and chromosomes in tumour cells were described in the late 1880s. Von Hansemann, in the 1890s, suggested that tumour cells develop from normal cells because of a tendency to mal-distribution and other changes of chromosomes occurring during mitosis. In the first decades of the 20th century, Mendelian genetics and "gene mapping" of chromosomes were established, and the dominant or recessive bases of the familial predispositions to certain tumour types were recognised. In the same period, the carcinogenic effects of ionising radiations, of certain chemicals and of particular viruses were described. A well-developed "somatic gene-mutational theory" of tumours was postulated by Bauer in 1928. In support of this, in the next three decades, many environmental agents were found to cause mitotic and chromosomal abnormalities in normal cells as well as mutations in germ-line cells of experimental animals. Nevertheless, mitotic, chromosomal, and other mutational theories were not popular explanations of tumour pathogenesis in the first half of the 20th century. Only in the 1960s did somatic mutational mechanisms come to dominate theories of tumour formation, especially as a result of the discoveries of the reactivity of carcinogens with DNA, and that the mutation responsible for xeroderma pigmentosum causes loss of function of a gene involved in the repair of DNA after damage by ultraviolet light (Cleaver in 1968). To explain the complexity of tumourous phenomena, "multi-hit" models gained popularity over "single-hit" models of somatic mutation, and "epigenetic" mechanisms of gene regulation began to be studied in tumour cells. More recently, the documentation of much larger-than-expected numbers of genomic events in tumour cells (by Stoler and co-workers, in 1999) has raised the issue of somatic genetic instability in tumour cells, a field which was pioneered in the 1970s mainly by Loeb. Here these discoveries are traced, beginning with "nuclear instability" though mitotic-and-chromosomal theories, single somatic mutation theories, "multi-hit" somatic theories, "somatic, non-chromosomal, genetic instability" and epigenetic mechanisms in tumour cells as a background to the chapters which follow.

Key words: Cancer, carcinogenesis, chromosomes, genetic instability, historical, nuclei.

Introduction

There are several excellent histories of the study of cell biology and of tumours [1–14], which give coverage of most of the various aspects of cells and this disease process. However, no perfectly satisfactory account of the history of investigations of the relationships between the morphological features and aetiological factors of tumours is available, especially in terms of the

genetic theories of the pathogenesis of tumour formation. Essentially, the history of the investigation of mutations in tumour cells is characterised by an early attempt by von Hansemann to provide a theory that embraced morphological abnormalities and chromosomal changes. This theory was rejected and largely forgotten, but was followed, over a period of approximately 70 years, by slow recognition, first of single mutations in tumours, then of multiple mutations in tumours. Recently, using molecular methods based on the polymerase chain reaction, so many mutations in tumour cells have been demonstrated that only acquired somatic non-mitotic, non-chromosomal genetic instability, together with alterations of gene expression, appears to provide a likely explanation. This chapter sketches the major milestones of tumour genetics, with detail given mainly when it has not previously been published in English.

The nature of the essential abnormality of cancerous tissues has been discussed since the disease was recognised as a process separate from inflammatory disorders, by the Hippocratic School in the 6th century BC. However, studies of tumours according only to clinical features and macroscopic appearances, by Greek, Roman, Arab, and medieval and Renaissance Europeans led to concepts and schemes of classification of tumours that were largely arbitrary and unhelpful [2, 8, 9, 12].

Beginning in the 16th century, various forms of microscopes were developed in Europe. The compound form is said to have been invented by the Janssen brothers in about 1590 [15, 16]. Early compound microscopes suffered especially from poorly made glass, as well as chromatic and spherical aberration, and were not necessarily more useful than the simple microscopes of the period, for example, those of Leewenhoek (1632–1723) [15, 16]. The progress of microscopic discoveries in the 17th and 18th centuries was achieved mainly through gradual improvements in the making of the glass for lenses. Thus, early in the period, lacteal and lymphatic vessels were described and only later were red cells in blood and "globules" in lymph, as well as fibres in many tissues discovered [4]. One of the last, but important discovery using a simple microscope with a non-achromatic lens (see below) was that of the nucleus by Robert Brown in the early 1830s [17]. Regarding tumours, one view in this period was that tumours derive from particular ("plastic") types of lymph, perhaps through a process of coagulation [8, 12, 18].

Achromatic lenses and the work of Johannes Müller

The development of cell theory probably could not have occurred without the development of better microscopes. These were developed concurrently by microscope makers in England, France, Germany and Italy [15, 16]. Attempts to make lenses with reduced chromatic aberration by combining glass of the crown and flint types began in the 18th century [16]. However, only in the mid-1830s, and because of the theoretical advances in optics provided by J.J. Lister

(1769–1869, father of Lord Lister [13]) in 1830, were useful achromatic lenses manufactured [16]. Microscopes with these lenses could magnify 500 times, and a wealth of scientific discovery followed. Within a few years, it was appreciated that cells are the basic living units of the body, and which, by multiplying and secreting extracellular materials, form all the tissues, i.e. the "Cell theory". Wolff [2] gives priority to Raspail (a French histochemist, 1794–1878 [13]), both for this idea, and for being first to use the word "cell" for the microscopic structures now recognised as such. The same idea, however, was propounded in detail by Schleiden (1804–1881) (for plant cells) in 1838 [19] and by his friend Schwann (1810–1882) (who gave generous credit to Schleiden) for animal cells in 1839 [20].

Schwann did this work while he was an assistant to Johannes Müller (1801–1858), Professor of Anatomy and Physiology at the University of Berlin, who was perhaps the most remarkable scientific teacher in modern history. This is because his students included not only Schwann, His (1811–1887), Henle (1809–1885) and Kölliker (1817–1905) [13], but also Virchow (1821–1902), who was the major proponent of cellular pathology (see below), Helmholtz (1821–1894), who propounded the law of conservation of energy as well as contributing to optics and acoustics, and Wilhelm Wundt (1832–1920), who founded experimental psychology. Another of Müller's students was Brücke (1819–1892), who in turn was a major influence on Sigmund Freud (1856–1939) [21].

Although Müller's research interests were mainly in neurophysiology, in 1838 he published [22] studies of the cells and their nuclei in tumours using a Schiek microscope of the latest type. Müller [22] documented the variable sizes and shapes of cells and nuclei, not only between tumour types, but also between cases of the same tumour type, and also among the cells of individual tumours. In so far as nuclei were understood to probably contain at least some of the hereditary material of cells [23], the finding of variability of nuclear morphology in cells might be considered the original observation of a form of hereditary/genetic instability in tumour cells, even if it was not appreciated as such at the time.

The beginnings of histology, cell biology and the cellular pathology of tumours

The improved techniques for microscopy (above) rapidly resulted in understanding of the general structure of animal and plant tissues, as well as their embryological development, in the current senses [4, 9–12]. These basic observations identified several major issues which were to dominate the study of cell biology for the next 40 years. The views of Virchow, who was Professor of Pathology at Berlin 1856–1902, are given prominence in the following discussion, because he was the most prominent pathologist of the 19th century, and strongly influenced the entire discipline of pathology in that era.

1. How do cells arise? Schwann [20], Müller [22] and Kölliker [24] thought that cells could arise spontaneously in some specific (but invisible) type of interstitial fluid, which they referred to as "blastema". Müller in particular thought that the process involved first crystallisation of nuclear material in the "blastema" and, second, the aggregation of cytoplasm around the crystals [22]. Müller thought cancer cells arose from particular cancerous invisible particles, which he called *"seminum morbii"* [22]. The alternative view, being that cells must always develop from pre-existing cells was espoused by Raspail, Remak (1815–1865) and, most famously, Virchow. For accounts of this well-documented controversy, which lasted into the late 19th century, see especially [5, 8–12] and references therein.
2. How do cells and nuclei divide to generally provide for daughter cells, which are the same as each other and the mother cell? Even with the best achromatic lenses, it was not possible in histological preparations to see any more detail of nuclear division than a division of nuclear material into two parts, followed by division of the cell, and this matter was not resolved in this period (see below).
3. To what degree do the embryological origins of the cells determine their ultimate morphology? Histological studies of embryos had led to suggestions, particularly by Remak and His [1, 3, 4, 8–12], that there are two or three basic embryological layers, from which adult cell types derive. Tracing the developmental "pedigrees" of cell types in adults was a major activity in embryology until the 20th century [1, 3]. A part of the stimulus for these studies was the urge to further investigate earlier observations of Caspar Wolff (1733–1794), von Baer (1792–1876) and Oken (1779–1851) [3, 4, 25, 26], who had shown that the phases of embryonic development which occur in one species resemble, albeit temporarily, the adult forms of "simpler" species that are "lower" or "earlier" in the evolutionary tree. The results of these histological studies generally supported the earlier observations, and were popularised as the saying "Ontogeny recapitulates Phylogeny", especially by Haeckel (1834–1919) [4, 26–28]. These studies formed part of the well-documented struggle (broadly from 1858–1940) to establish scientific evidence for and against Darwin's theory of evolution [29–33].
4. Can cells undergo changes of mature morphological type in adults? Or are they always faithful to their original lineage? Virchow pointed out that chondrocytes and osteocytes associated with the callus of healing of fractures of bone arise from basic connective or "supportive" tissue [34] (*Bindegewebe*: for a useful note on the English translation of this word, see Translator's Notes in [25]). For these changes of cell type, Virchow used the phrase "histological substitution" in the second edition of his "Cellular pathology" (1858) [34], but used "metaplasia" for the same process in the fourth edition of "Cellular pathology" in 1871 [35] and in a later article in 1884 [32] (see also [36]). However, the same authors who supported fixed continuities of embryological layer to adult cell type also tended to support a view of fixity of cell type in adult tissues (see above).

5. As an extension of the fourth issue above, can cells of tumours come from a single "tumour precursor cell" in connective tissue or from the more specialised cells of each type? Again, Virchow insisted that interchanges of cell types are common phenomena and that cancers do not come from epithelial cells, but rather from particular cells of the *Bindegewebe* [34, 35]. Eventually, the opposite view, especially in regards to the derivation of carcinomas from epithelial cells only, came to be most widely held, due especially to the work of Waldeyer (1836–1921) and Thiersch (1822–1895) [8–12]. The present view is that some, but not all, cell types or their local "stem cells" retain some ability to adopt different directions of histological differentiation under particular circumstances [37].
6. What is the stimulus to the excessive growth of tumours? By the mid-19th century, ideas of "blastemas" and "plastic lymph" had been abandoned, and Virchow's [34] suggestion that a chronic local "irritation" must be the first step of tumour formation was popular. Thiersch, in 1865 suggested that a local over-nutrition of tissues might cause excessive growth [2], and Bol, in 1876 proposed that tumour cells derive their growth in some way from some influence of embryonal-like mesenchymal cells at the site of tumour formation [2]. Cohnheim's idea [38], published in 1882, was that the cells of tumours are essentially embryonal in nature, being "left-over" embryonal cells. In this way, carcinomas are epithelial because they arise from "left-over" embryonal epithelial cells. To account for the sudden activation of these "dormant" embryonal cells, Cohnheim suggested a mechanism of local hyper-nutrition [39]. Ribbert (1855–1920) initially invoked embryonal rests as the source of tumour cells [2], but later thought that normal adult cells might be stimulated to grow entirely because of a loss of normal local inhibitory "tissue tension" [39] (reviewed in English [2, 40, 41]).
7. What is the mechanism of the nuclear pleomorphism to tumours? None of the various mechanisms of tumour formation (above), however, provide an explanation for nuclear pleomorphism in tumour cells. Perhaps stimulated in part by this consideration, much effort was expended in the late 19th century on the (intranuclear-) parasitic theories, for which the best evidence obtained was that the intranuclear irregularities of tumour cells resemble either the bodies of, or the effects of, parasites. (Wolff [2] devotes 150 pages to these theories. Shimkin [8] gives a useful table.) These were very popular up to the early 20th century and were mentioned by Ewing as late as 1940 [42], although the evidence in their favour was entirely morphological, and conversion of normal cells to cancer cells by the intranuclear "cancer parasites" was not achieved.
8. In the most general sense, what is the relationship of disease processes to normal physiological processes? The Ancient Greeks and Romans, especially Galen, held that all diseases, excepting trauma and parasitic disorders, are due to "imbalances of (normal physiological) humours" and hence are endogenous in origin [2, 5, 8–12]. Müller [23] held that diseases are "abnormal physiologies" and Virchow repeatedly and strongly stated the same view [9, 43, 44]. To give just two quotations, in 1855 [45], Virchow stated "All pathological for-

mations are either degenerations, transformations, or repetitions of typical physiological structures." In 1877 [46], Virchow repeated the same opinion "… I must strongly emphasise that pathological formations never develop beyond the physiological possibilities of the species." One object of Virchow's position was to distance himself from all non-mechanistic ideas in pathology, such as the involvement of "vital energies" and "special life forces" [43]. An effect of his views, however, may have been to encourage his students to find physiological processes that paralleled any new phenomenon of disease they might wish to describe (see below).

Mitosis, chromosomes and Von Hansemann's theory of cancer

In the next period, however, microscopical techniques were introduced, which allowed the discoveries that form the basis of our much of our current histological and pathological understanding. From the 1870s, a variety of non-optical improvements were made to histological techniques, including new fixatives, paraffin embedding, better section cutting and better stains (aniline dyes, followed by haematoxylin and eosin) [15, 16]. In addition, "optically homogenous" oil immersion, with appropriate new types of lenses, and achromatic substage light condensers were introduced by Abbe (1840–1905) in the late 1870s at the Zeiss factory in Jena [16]. In the 1880s, Abbe invented apochromatic ("away from colour") lenses, which were composed of glass with various novel additives, especially borate. These lenses were released commercially in three series, in 1886, 1888 and 1894 [16], so that optical resolutions (0.25 μm), close to the highest that can be achieved using visible light, were obtained.

These improved methods were applied to the events of cell division, as outlined in [3, 4, 9–14], and the condensation of chromatin into "threads" (also termed "loops", "filaments" and later "chromosomes", or "nuclear segments" [1]) prior to nuclear division was discovered by many authors, including Strassburger, Waldeyer, Flemming, Boveri, van Beneden and others [1, 3, 4, 14].

Von Hansemann's first paper on cancer (1890)

In relation to tumours, several authors (references in [47]) commented on the abnormalities of chromosomes of tumour cells, but only Klebs [48] considered that these might have any pathogenetic significance for tumour formation. Most notably, however, the topic was taken up by von Hansemann (1858–1920) in 1889, who had graduated in Medicine only a few years before (*Staatsexamen* 1886) and was, at the time, the junior (third) *Assistent* in Pathology to Virchow in Berlin. Von Hansemann used all of the new techniques, and produced a remarkable synthesis of the cell biological principles of the time and his own observations to create the first chromosomal theory of

tumour formation. The general outline of his concepts appeared in his first paper in 1890 [47], while later articles and two books [49, 50] contained extensions and modifications of his ideas, as well as responses to the frequently negative comments published by other authors.

The first paper (1890) [47] is difficult to understand for two reasons. First, von Hansemann probably felt that, before he could elaborate a notion of cancer, he had to describe a normal biological process which, when mildly abnormal, would produce appearances resembling those of cancer (perhaps under the influence of Virchow, see above). Thus, it was probably not enough, in 1890, for von Hansemann to observe that, if chromosomes carry the genetic material of the cell, and are abnormal in cancer cells, then the abnormal chromosomes are probably the cause of the abnormalities of cancer cells. Put another way, von Hansemann possibly had to satisfy Virchow's somewhat abstract notions of disease pathogenesis (see above) and find a whole analogous system of biological process, which in some way resembled many if not most of the tumourous phenomena. Second, at the time von Hansemann wrote the paper, the differences between the chromosomal replications and divisions in meiosis and mitosis were not recognised, nor were the numbers of chromosomes in human adult cells or gametes known. Furthermore, the individuality of chromosomes was only one theory among many at the time, and "genes" and "gene maps" lay in the future.

At the beginning of this paper [47], von Hansemann discussed ideas of the variability of amounts of chromatin in cells generally. He then noted that injection of the chromatin of a sperm is an important aspect of fertilisation of the egg, and that the amount of chromatin increases and decreases in cells associated with spermatogenesis in testicular tissue. Von Hansemann then observed that in tumour tissue, increases and decreases of chromatin in tumour cells occurs, and that the smallest nuclei appear to become degenerate. This last process that von Hansemann observed, seems to be in some way analogous to the expulsion of the polar bodies (referring to them as did Hertwig [25] as *Richtungskörperchen*, which later came to be used for "centrioles") from the developing egg in the ovary. Next, von Hansemann discussed asymmetric distribution of chromosomes in mitosis as the main mechanism of the formation of small nuclei in some detail. In the next section of the paper, he reviewed theories of cell heredity as they were known in 1890, mentioning especially the ideas of Weismann and Naegeli, in relation to "quantitative" and "qualitative" asymmetries of cell division (not nuclear division) during formation of the blastula. This discussion led to consideration of the progress of differentiation of the cells in the early embryo, with two "pivotal" statements being made, to try to link differentiation, autonomy and growth. They were:

1. "With every further qualitative work division, the cells lose the capability to exist autonomously."
2. "With every new generational phase, a changed growth energy ["nutritional, formative, and functional activity" (Virchow)] takes place which often

manifests itself in a change in direction of growth." (The original was in bold emphasis).

Von Hansemann then went on to discuss the capacities for differentiation of adult cells, as demonstrated by the results of transplantation experiments, before returning to oogenesis. In this part, he used the view of Weismann (references in the paper) that the process of development of the ripe egg in the ovary is one of transition from a cell which is of a differentiated (germinal epithelial) type, to one which has no capacity to subsequently "differentiate" (of course unless fertilised), and thus should be considered as completely undifferentiated. Von Hansemann therefore described the change which the ripening egg has undergone as *Entdifferenzirung*, which is best translated as "dedifferentiation", in the sense of a cell having been differentiated, but being no longer so. (In German, *ent-* is usually used for "the condition following the removal of something", for example when a church is deconsecrated, as opposed to unconsecrated.) Von Hansemann then named the separate phenomenon of the process of differentiation of the egg after fertilisation (i.e. all embyronic development) "prosoplasia" and named the dedifferentiation of the ovarian germinal epithelium to the ripe egg, "anaplasia".

In the remainder of the paper, von Hansemann discussed further the functions and "differentiation" of cells, and the role of changes of chromatin. In one passage, von Hansemann justified all of this background material with the words (which possibly relates to the Virchovian position of "every disease process is an abnormal physiological process', see above):

"Touching on this theme may be justified by the fact that, in so far as I have wished to draw any conclusions from my observations on epithelial cancers, I had to take a position *vis à vis* a sequence of biological questions."

He then cited transplantation experiments with embryonic tissues, to emphasise that tumour cells are not only different to embryonal ones (and thus in opposition to the "embryonal rest" theories of Cohnheim, see above) but also possibly have an egg-formative character. From all of this, von Hansemann's concluding sentence in the 1890 article [47] can be comprehended:

"Thus, as far as anaplastic cells are concerned, they must not be confused with embryonal ones, in fact, there is a clear contrast between the two, and the embryonal cells begin where the anaplasia ends, with the egg."

Von Hansemann's later works

Shortly after von Hansemann's article [43], the true nature of the chromatin ejected from the ripening egg (surplus haploid nuclei) was recognised [3, 4, 51]. Also, the model of differentiation involving quantitative changes of chromatin was abandoned [3, 52], Von Hansemann in later publications [49, 50] gradually abandoned the "egg-formative" "physiological prototype process" part of his theory, but retained the essence of the chromosomal mechanism of

tumour formation. He described the phenomena of progressive disturbance of mitoses in tumour cells and of increasingly abnormal morphology and characteristics of chromosomes, including their "lysis", "stickiness" and other changes, in tumour cell nuclei in association with increasing clinical aggressiveness [49, 50]. This cellular process of mitotic and chromosomal instability is currently often referred to as "clastogenesis" [53, 54] and the associated behavioural process as "tumour progression" [55, 56]. He also discussed the relationships of the chromosomes to cell function, and provided numerous other, and still-relevant, insights into the relationship between the morphology of tumours and their pathogenesis.

Contemporaneous responses to von Hansemann's theories; Boveri's theory

At the time, however, his ideas were rejected. Most authors, for example R.C. Whitman [57] (at the University of Colorado, not to be confused with C.H. Whitman, Director of the Woods Hole Marine Biological Institute, Maine) did not understand von Hansemann's ideas, or confused them with the concept of "backward reversal" of embryonic differentiation (as originated by Bol in 1876, see above, and [58]), or rejected them out of hand as impossible [39].

Boveri (1862–1915), a biologist who had previously made great contributions to the understanding of mitosis, published a volume on the origin of tumours in 1914 [59]. In this book, Boveri mainly suggested that quadipolar mitosis might be a significant mechanism in the induction of tumourous behaviour in cells. His theory was poorly considered in terms of pathology, and appears to owe more to von Hansemann than he (Boveri) admitted. Thus, von Hansemann is mentioned by name (pages, 6, 24, 67, and 108 of the English translation of Boveri's volume [59]) but never by citation of Hansemann's articles or books. Moreover, Boveri wrote (pp 23–24 [57]) "The cell of a malignant tumour is accordingly (and here I take up again the idea of Hansemann) a cell with a definite (*sic*) abnormal chromatin complex." However, on p 111 Boveri [59] stated "The essence of my theory, is not abnormal mitosis, but in general, a definite (*sic*) *abnormal chromosome complex* (original italics)."

Because von Hansemann had already, by 1914, described a variety of non-mitotic abnormalities of chromosomes in tumour cells (see above), Hansemann's priority seems to have been overlooked. Boveri [59] seems to have used ideas which were very similar to those of von Hansemann, except for specific reference to quadripolar mitosis.

"Dedifferentiation" and "anaplasia" remain in use

Von Hansemann's terminology of "dedifferentiation" and "anaplasia" were extremely successful, and remain in use today. This is because the classifica-

tion of tumours prior to 1890, was of "homologous" (like the adjacent normal tissue) and "heterologous" (unlike the adjacent normal tissue). This classification, which dated from Laennec in 1804 [2] and was used by Virchow [34] and others, did not permit any intermediary types, while von Hansemann's concept (dedifferentiation/anaplasia) was of a process which could occur in grades and degrees [2]. Von Hansemann's views were based on actual histopathological phenomena, which were being more and more widely documented in diagnostic histopathology throughout the world from the 1890s onwards, using the new techniques and microscope lenses (see above). "Dedifferentiation" and "anaplasia" entered the medical lexicon, where they remain firmly to this day.

Reappraisal

Perhaps correctly, von Hansemann's notion of "anaplasia" (with its component of either the correct "dedifferentiation" or the incorrect "undifferentiation" concept of the cell) has been discounted. However, from the perspective of the 21st century, we can see that his basic idea of chromosomal disorder as the basis of tumour formation may well be valid, and current aspects of chromatin and chromosomes in cancer are the subjects of chapters 2 and 3 in this volume.

On the basis of all of this, it would appear that von Hansemann may deserve more recognition as a contributor to genetic theories of tumours and oncology generally, than he is currently awarded.

Early 20th century studies of carcinogenesis in relation to the cell biology of cancer

Hereditary factors and Mendelian genetics in tumours

In the 18th and early 19th century, many authors, including John Hunter [18] considered that families can inherit predispositions to cancers, generally in keeping with "humoural/diathesis" concepts of disease [60, 61]. Detailed studies of families to test this were undertaken and continued into the 20th century, for example, by Warthin [62]. A few familial predispositions, however, were known to be to tumours of one type only. For example, von Recklinghausen's neurofibromatosis was known to be a familial disorder as early as the 1880s [63]. Only after the application of Mendelian genetics to human diseases was the nature of these predispositions established. Thus, familial polyposis coli was found to be autosomal dominant, and xeroderma pigmentosum was shown to be autosomal recessive in their respective genetic transmissions by the 1920s [60, 61].

At the beginning of the 20th century, inherited predispositions to tumours were investigated experimentally. Numerous breeding programmes of experi-

mental animals were conducted, especially in the UK and the USA [8], and it was found that, indeed, the inbred offspring of animals with certain tumour types were more liable to the same tumour, but not to tumours generally. Maud Slye (1879–1954) thought that the results of such studies showed that human tumours are of a "recessive" type, but this was not supported by other workers, notably Little (1888–1971) [8, 64].

Another line of investigation was the transplantability of experimental tumours between members of the same species, and across species [8, 65–69]. Initially, transplantation experiments were conducted in the investigation of infectious theories of cancer according to the "Koch's postulates" used for infectious diseases. No transfers of disease to normal recipient cells by tumour tissue occurred. Subsequently, transplantation of tumours was used to study the hereditary factors associated with the susceptibility of the recipient animals to tumour "take", and claims were made that this was dominantly inherited [66]. Later, the effects of immunological reactions to these transplants were recognised, and it transpired that most reactions appeared to be due to the recipients' reactions to the species-related antigens of the donor, rather than any reactions to tumour-specific antigens [67]. Other studies were directed at factors associated with metastasis, which was similar to von Hansemann's feature of "capacity of the tumour cells for independent existence", or "autonomy" (see above). Leo Loeb [68] in 1937 considered that the major determinants of growth of transplanted tumours include immune reactions of the host, but also that the "growth energy" or "growth momentum" of the transplanted tumour tissue is important. Because growth rate of tumours and degree of dedifferentiation are often related, and rapidly growing tumours may access host blood vessels faster than the host tissues can react with fibrosis, this may be an adequate explanation of tumourous "greater capacity for independent existence" (see above).

Despite this unsatisfactory situation concerning the actual significance of heterotypic survival, these transplants of tumours provided useful models of cancer for the study of various aspects of cancer, not the least of which was anti-cancer therapies. The distinction between degree of "autonomy" and "susceptible to immunological rejection by the recipient animal" could not be made easily until the advent of the nude mouse in the 1980s [70].

Chemical agents

Although workers in certain occupations had been known to be susceptible to cancers in the 18th century, the chemical or physical basis of these were not widely considered. This may have been due in part to the fact that these diseases were still considered to be due to some generalised imbalances of humours, and thus direct action of these agents on cells were perhaps not understood to be relevant. Snuff cancer was described in 1761 by John Hill, chimney-sweep's cancer 1775 by Percival Pott and pipe smoker's cancer (of

the lower lip) by Soemmering in 1795 [8]. Arsenical compounds were described as causing skin cancers in animals in 1822 and in humans in 1888 [8, 12]. Tar and paraffin cancers were described by von Volkmann in 1875 [2, 5], and mine worker lung cancers were recognised in 1879 [8].

In 1895, aniline dyes were found to cause urinary tract cancers [8]. In the early 20th century, coal tar was proved to be carcinogenic in rabbit skin by Ichikawa and Yamaguchi [8, 12], although Hannau had failed to produce such lesions by repeated application of coal to the scrota of dogs in an experiment in the 1880s [2].

In 1930, the first pure carcinogenic hydrocarbon was isolated from coal tar by Kennaway and his group [71], allowing for detailed studies of the biological effects of these compounds, with so-far-unsuccessful attempts to establish relationships between their chemical structure and carcinogenic potential [72, 73]. The ability of some chemical carcinogens to cause germ-line mutations in experimental animals was shown in the late 1920s and 1930s [6].

Meanwhile, the number of categories of known carcinogens has expanded to include aromatic amines, nitrosamines and alkylating agents [8, 74]. In the 1960s, it was also found that chemical carcinogens can cause strand breaks in DNA in cells [8]. Some problematic inconsistencies between the chemical activities, including degrees of DNA "adduct" formation and the carcinogenic potencies of various chemical agents were documented early in these studies (for a recent discussion see [75]).

Aspects of chemical carcinogenesis are the subject of chapters 4 and 5 of this volume.

Physical agents

Ultraviolet light was discovered in 1801 by Rittner, who noted the ability of a component of sunlight beyond violet light to darken silver chloride [76]. Sunlight was suggested to be the cause of sailor's cancers by Unna in 1894 and ultraviolet light was shown to be able to cause skin cancers in white mice in 1928 [8]. Chapter 6 of this volume deals with current issues in ultraviolet carcinogenesis.

X-rays were discovered in 1895 by Roentgen (1845–1923) [8, 12], and uranium salts were shown to emit gamma rays in 1896 by Becquerel (1852–1908) [8, 12]. The former were understood to cause skin cancers as early as 1902 by Freiben [8, 12] and isotopes taken internally were reported to cause bone cancers in 1925 [8]. Experimental induction of germ-line mutation in *Drosophila* by X-rays was demonstrated in 1928 by H.J. Muller [6], and it was established in the 1930s that irradiation causes chromosomal lesions in cell cultures *in vitro* [77]. Chapters 7 and 11 deal with current aspects of radiation-induced carcinogenesis.

Infectious agents

Numerous parasitic theories of neoplasia were proposed from the 19th century on the basis of structures suggested to be these parasites in the cytoplasm and nuclei of tumour cells (see above). The association of bilharzia and bladder cancer was suggested as early as 1889 [8].

Peyton Roux, in 1911, reported that a tumour of fowls could be due to a transmissible, filterable agent [8, 12], and subsequently Shope reported that a filterable agent could transmit papillomata of the skin of rabbits [78]. Subsequent developments in the field showed that oncogenic viruses may be of either RNA or DNA type [79]. Current aspects of viral oncogenesis in relation to the host genome are discussed in chapter 8 of this volume.

Tissue processes as "targets" in carcinogenesis

While the focus of this chapter has so far been on individual cell, there remain the problems of intra- and intercellular controls of tumour cell behaviour, and the overall concept of cancer as a disorder of a single fundamental biological process. The concept of tumours arising by a disturbance of a normal tissue process has been popular since Virchow in the middle of the 19th century (see above).

Abnormal hyperplasia has been recognised as a frequent preliminary morphological change in tumours since Virchow (see above), and is well documented, for example, in tumours of the human endometrium [80], in experimentally induced lesions of the skin [81] and in the breast of mice [82]. One of the major features of tar-induced experimental skin tumours is that a phase of reversible epidermal hyperplasia occurs, as stressed by several authors [82–84].

Abnormal wound healing was proposed as the basic process of cancer by several authors, for example, Haddow [85], and later workers suggested that local hormones (for example, "chalones" [86]) that control the cell proliferation associated with healing may mediate these abnormal responses.

Abnormal "differentiation" as the fundamental process of carcinogenesis continues to be extensively investigated, although the concepts of modern authors are distinct from the proposed "loss of differentiation" (*Entdifferenzierung*) described by von Hansemann (see above). Harris [87] in 1990, reviewing differentiation and tumour formation, noted the "ancient question" of whether a tumour grows rapidly because it does not differentiate, or does not differentiate because it grows rapidly. Harris [87] conceded that this association could arise if a separate cause has both effects, and the present author has shown that, among the various human tumours, examples are to be found in which lack of differentiation and high growth rate are not correlated at all [88]. For some recent reviews of notions of differentiation as a primary event in cancer see [89–91].

The role of the mesenchyme in tumour formation (foreshadowed by Virchow, and espoused by Ribbert, see above) has had several recent supporters, especially those concerned with epithelial-mesenchymal interactions in normal biology [92–94]. Epithelial-connective tissue lineage infidelity is a separate issue, which has been revived (since Virchow) and referred to as "transdifferentiation" [95, 96], epithelial-mesenchymal "plasticity" [97], epithelio-mesenchymal transformation [98] and epithelial-mesenchymal transition [99].

Another cellular process relevant to tumours, which is currently undergoing intensive current study, is apoptosis [100]. This process is a defence of the body against tumours, in that, under normal circumstances, all cells except the permanent stem cells of tissues, ultimately die and in the case of epithelial cells are shed rather than being resorbed. Thus, cells which suffer mutations during transit amplification of epithelia are eliminated by shedding. These issues are discussed in chapter 9 of this volume.

Nineteenth century ideas of the involvement of blood vessels in the pathogenesis of tumours by way of a "nutritive" growth-stimulating effect are mentioned in chapter 3. The role of angiogenesis in tumour formation is a new concept, and is discussed, in terms of current investigative techniques, in chapter 10 of this volume

Somatic mutation in tumour cells and the number of mutations per cancer cell

"Single-hit" somatic mutation theories of tumours

Although the idea of alteration of hereditary material of adult cells as the basis of cancer is implicit in Virchow's idea of hybridising of somatic cells [2], in parasitic theories [2, 8], and in von Hansemann's concepts of altered chromosomal composition (see above), in the first half of the 20th century the ideas were not universally accepted in relation either to spontaneous human tumours, or to chemically or physically induced experimental tumours.

Nevertheless, with the rediscovery of Mendel's work in general, interest in the alteration of genetic material re-emerged. De Vries, in his "Mutation theory" (1902) [101], reviewed evidence of alteration of the genetic material in somatic cells of plants, using the term "vegetative mutations" of which "bud variations" comprised one type. The involvement of alterations of somatic cells in this variation was recognised by Bateson [102], and the term "somatic mutation" was used by Tyzzer in 1916 [103], and by Whitman in 1919 [57].

Probably the most extensive early discussion of "mutation" in relation to tumours was that of Bauer [60]. Because no translation of the work of this author has apparently been published, some detail will be given here. Bauer [60] provided a well-constructed volume, in which various biological phenomena, experimental results, and human diseases were considered. Based on

the work of germ-line mutation in *Drosophila* by Morgan and co-workers [104], Bauer observed:

"Considering the problem of tumours, it is of decisive importance whether such mutations, whose occurrence in the germinal cells has been proved a thousand-fold, can also appear in the body cells. In this respect, one can say *a priori* that it would have to appear uncommonly striking if the fundamental biological process of gene alteration were possible only in the chromosomes of the primordial germ cells (*Urkeimzellen*) and impossible in all the other cells."

Bauer [60] went on to discuss the botanical evidence in favour of somatic mutation as a possible phenomenon, before discussing the possibility that human developmental lesions that occur singly as an uninherited condition (such as isolated exostosis) could be due to somatic mutations during embryonic, foetal or histological development. This, he pointed out, could be particularly true if multiple lesions of the same type occur as an inherited condition (e.g. inherited multiple exostoses). Let Bauer [60] speak for himself:

"In contrast to these systematised forms of disease, there are also locally limited forms of the same fundamental disturbance, e.g. partial albinism of one eye, solitary exostosis, individual bone cysts, unilateral cystic kidneys, etc.

These forms, which in contrast to the generalised forms, are locally limited, are characterised by the fact that: (1) they virtually always appear singly, (2) they are never inherited or hereditable, and (3) morphologically, they are essentially identical to the generalised forms.

In all these and similar cases, medicine found itself embarrassed with respect to their aetiological interpretation. We do not wish to make fun of medievally naïve ideas which explained such systemic illnesses, if they appeared in the multiple manner, by general pressure, and if they appeared solitarily, by local pressure of a constricted amniotic cavity, and explained the fact of hereditability of the ailment by the hereditary tendency to constriction of this cavity. We must, however, reiterate that there was no satisfactory interpretation for the facts just given. Mostly, one avoided the difficulty by contenting oneself with the observation that the relevant disease appears now multiple and hereditary, and now solitary.

Thus, in all these malformations, with their identical essence, which sometimes appear generalised, and sometimes are locally limited, we are dealing with the same process as in the bud mutations of a plant. A mutation when it appears in the germinal cells is hereditable, and in the bearers of the mutation, then spreads to the entire tissue system dependent upon it. The same mutation when it appears in the body cells has effects that are locally limited; only in the cells which are descended from the cell first are mutated. Thanks to the change occurring in the same gene, the mutation causes the same morphological picture, but is naturally never hereditable as it, being a mutation of somatic cells, does not belong to the germinal line."

Bauer [60] made numerous other relevant observations, but this early work has been cited infrequently in recent years, and is currently neglected.

In relation to the mutations and the development of tumours, some chemical carcinogens were found to be germ-line mutagens by many authors [6, 105]. Nevertheless, the idea of somatic mutation as an important direct effect of carcinogens was resisted especially by Berenblum [106, 107], Foulds [82] Willis [84] and Burdette [108]. Another example was Earle who, in 1943 [109], reported that normal cells cultured *in vitro* can change into cells that can form tumours when they are injected back into the animal of origin. He did so without mention of mutation, just as later reviewers of this topic into the 1960s omitted discussion of mutation [110, 111].

Only the discovery of viral oncogenes by Huebner and Todaro in 1968 [112], followed by later documentation of endogenous cellular oncogenes/growth factors, established mutation as a widely held basis of tumour formation (see reviews [113–115]).

Morphological considerations in relation to "single-hit" theories

Despite the above, a stumbling block of all chromosomal and single mutational theories of tumours has remained the morphological variability of tumours. This was most eloquently argued by Willis in 1948 [84], who objected to the theory that a small number of mutations are the basis of carcinogenesis, mainly because each effective mutation should rapidly produce a sharply distinct new population of cells, whereas, in most instances, experimental tumours arise slowly through prior hyperplasia-like abnormalities. Furthermore, in human tumours, lesser degrees of cytological abnormality are often seen at the margins of the tumour, rather than a sharp demarcation of the tumour from normal cells (for a more extensive discussion see [116]). Willis' opinions have been echoed in the more recent literature [117] and they are probably among the reasons that recent textbooks of pathology [118–120] indicate that the nature of the abnormality of cancer cells is essentially unknown.

"Multi-hit" models of somatic mutation

From the 1920s, models of carcinogenesis involving more than one mutation in a single cell were proposed as the basis of tumour formation. This was the basis of clinico-pathological studies of human tumours, on experimental investigations in a variety of research areas and on epidemiological studies.

In human studies, the views of Bauer [60] are mentioned above. Another major contributor to this issue at the time was Lockhart-Mummery (in 1934) [121], who considered that each polypoid lesion in familial adenomatous polyps (FAP) must represent a somatic (mutational) event in an already mutant cell, thus foreshadowing "two-hit" theories of Knudson (see below). Lockhart-Mummery [121] stated "… some factor is inherited which renders certain tis-

sue cells of a particular organ unstable, so that mutation takes place, resulting in excessive mitosis of that particular cell (resulting in the adenoma)".

However, Lockhart-Mummery's view of the number of mutations required to cause a malignancy is somewhat unclear, because of his statement "Malignancy arises because of a second accident …associated with excessive proliferation". If "accident" here refers to a mutation, Lockhart-Mummery's theory is of "three hits" for malignancy.

Also on the basis of clinical studies, Nichols [122] in 1969, suggested that the tumours of neurofibromatosis arise by a second (somatic) mutation of the already mutant locus (the "n locus") of predisposed cells. Comings [123] elaborated a "general theory" of carcinogenesis, and made the same suggestion that tumours arise by mutation of both copies of "diploid pairs of regulatory genes" (i.e. both alleles of one gene – Comings used the term "gene" for allele in his paper). Applying concepts of "recessive oncogenesis" (see above), Knudson ([124, 125] reviewed [126]) has proposed that perhaps only two mutations are required for carcinogenesis generally.

However, single or even two or three mutations do not explain all the phenomena of more complex tumours, such as carcinoma of the colon. For this tumour, "activating" mutations of multiple genes have been proposed. For example, Vogelstein and co-workers [127, 128] proposed a five-step model involving a series of oncogenes. Another issue is the importance of sequential timing of these mutations. Fearon and Vogelstein (1990) [127] were of the definite opinion that "Accumulation, rather than order, is most important" in carcinogenesis.

More complex models, which go beyond a simple chains of activations, have been proposed recently [129, 130].

In experimental studies, once pure carcinogens were prepared in the 1930s (see above), it became possible to study possible synergistic effects of two or more carcinogens. The latter studies, undertaken especially by Berenblum and, independently, Mottram [73] showed that for many chemicals, tumour formation required the application of one particular type of chemical (the "initiator"), before the application of another particular type of chemical (the "promoter"). Neither chemical alone produced tumours, and no tumours were caused by the chemicals in the reverse sequence. It was recognised, however, that some chemicals ("complete carcinogens") could have both effects, and in some cases, the sequence did not matter ("co-carcinogenesis"). These data led to the popular "two-stage" concept of carcinogenesis with "initiation" and "promotion" being necessary phases of tumour formation [73, 74]. At the time, the mechanism of each of these processes was unclear, but later it was proposed that initiation represented a primary mutation of some particular "cancer critical" gene [131–134], and promotion was probably related to epigenetic phenomena [135].

In the context of these clinical and experimental findings, epidemiological investigations were carried out in the 1950s and 1960s, to try to confirm the

idea by statistical evaluation, on the basis of "population genetics" pioneered by R.A. Fisher [58, 136]. Several authors [137, 138] concluded that only a few somatic mutations might be necessary to cause a cell to become tumourous.

Armitage and Doll [139, 140] added tumour "progression" to the initiation/promotion model as a third stage of neoplasia. Progression later came to be considered to be caused by mutations [141] and by mutations arising from genetic instability in particular (see below).

Non-mitotic, non-chromosomal somatic genetic instability in tumours and replicative infidelity of DNA

From the foregoing, it seems that at least three broad types of "genetic instability" have been identified. First "mitotic instability", described essentially by von Hansemann (see above), results in mal-distribution of otherwise normal chromosomes to daughter cells, creating cells with imbalances of chromosomes. Second, there is "chromosomal instability", by which the chromosome structure (see especially [6, 14, 77]) is compromised, so that the chromosomes become more "sticky" or prone to breakage. This tends to be associated with "mitotic instability". A justification for this separation of "mitotic" and "chromosomal" phenomena is the existence of anti-tumour drugs, especially of the vinblastine group, which specifically disrupt the tubulin of the spindle fibres of mitosis [142]. The third type of genetic instability occurs without significant mitotic or chromosomal disturbance, and is associated with a reduction of the ability of a cell to faithfully reproduce its DNA sequence. The original information in favour of this concept (reviewed [143]) developed from the discoveries of bacteria that mutate more quickly ("mutator strains") than wild types. Later, it was found that, from a single tumour, strains of tumour cells could be grown that had different biological features and different karyotypes, indicating tumour "heterogeneity (see [143]). At approximately the same time, it was discovered that the mutant gene responsible for the increased rates of skin cancer among individuals with xeroderma pigmentosum encoded an enzyme associated with repair of DNA [144]. Subsequently, the idea has been supported by the discovery that a variety of inherited predispositions to tumours are associated with mutations of genes responsible for either repair of DNA or preservation of fidelity of replication (during S phase synthesis) of DNA [145]. In the 1990s, the application of methods based on the polymerase chain reaction (PCR, invented by Mullis [146]) led to the eventual quantification of the number of genomic events in the whole genome of carcinoma cells (by Stoler and co-workers in 1999 [147]). This study used directly *ex vivo* cells, and a new method of inter- (simple repeat sequence) PCR. The results [146] showed that the numbers of genomic events exceeded possible aetiological events and also the number of mitotic and chromosomal aberrations. Thus, the action of an acquired, non-

mitotic, non-chromosomal somatic genetic instability seems to be strongly supported. The basic ideas of this mode of carcinogenesis have been outlined and investigated particularly by L.A. Loeb since the 1970s (e.g. [148–151], and reviewed in [75]).

Aspects of genetic instability in tumours are discussed in chapters 11, 12 and 13 of this volume.

Abnormal gene expression in cancer

Despite the above ideas concerning the mutational basis of tumours, the possibility that an abnormality of gene regulatory mechanisms might contribute in a fundamental way to tumour cell pathogenesis remains. At present, many gene regulatory mechanisms are known, including chemical alteration of the DNA itself (especially methylation) and proteins and RNAs that act on (local) promoter regions. In addition, there are regulators of translation of RNA and factors that control the cell cycle (i.e. multiplication) of target cells. Aspects of these issues are covered in chapter 14 of this volume.

Conclusions

The history of the relationships of the morphology of tumour cells and their cellular genetics has involved numerous contributions from many apparently separate fields of biology. The broad cellular morphological observations, in terms of the variability of form, function and behaviour, were established in the 19th century due to the work of Müller, Virchow, von Hansemann and others. In particular, mitotic and chromosomal lesions noted by von Hansemann, although virtually ignored at the time, may find support in more recent karyokinetic studies of chronic myelocytic leukaemia, and some sarcomatous conditions. The genetic observations of most recent times, however, have revealed so much previously unsuspected genomic disturbance in tumour cells that some form of non-mitotic, non-chromosomal instability appears to be involved. Because all of these processes involve nuclear structures, and appear to be provokable by carcinogens, this volume has been designed to bring together chapters which deal with many aspects of these studies, and illuminate the latest aspects of these contemporary issues in cancer research.

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