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The Molecular Genetics of Soft Tissue Sarcomas

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INTRODUCTION

SOFT TISSUE SARCOMAS are a highly heterogeneous group of malignant tumours arising from mesodermal tissue such as fat, muscle and fibrous tissue [1]. The present article summarizes recent progress in understanding the genetic basis of these tumours. Progress has been limited by the rarity of soft tissue sarcomas (estimated to account for approximately 1% of all cancers in the U.K.) and by technical problems associated with karyotyping and establishing the tumours in culture. Analyses at the cytogenic and molecular genetic level are aimed at improving clinical diagnosis as well as contributing to a fuller understanding of malignant transformation in soft tissues.

GENETIC PREDISPOSITION TO SOFT TISSUE SARCOMAS

Hints at the involvement of genetic factors in soft tissue tumours emerged first from epidemiological studies which showed them to be associated with certain heritable cancer predisposition syndromes such as von Recklinghausen's neurofibromatosis, the basal cell naevus syndrome, tuberous sclerosis, Werner's syndrome and Li Fraumeni syndrome [2]. In Li Fraumeni syndrome the occurrence of childhood soft tissue sarcomas, in particular rhabdomyosarcomas, is associated with a high incidence of pre-menopausal breast carcinoma in close female relatives and, at a lower frequency, a spectrum of other tumours [2, 3]. Although familial predisposition is detectable only in a small minority of childhood sarcoma patients, the identification of such families provides the opportunity to carry out genetic linkage studies which may implicate one or more genes involved in both breast cancer and soft tissue tumours.

Soft tissue sarcomas (and also osteosarcomas) arise more commonly than expected in carriers of familial retinoblastoma [4]. The retinoblastoma gene (RB1) has been mapped to chromosome 13q14 and is inactivated by point mutation or homozygous loss in retinoblastoma [5]. In normal cells the RB1 protein is thought to in some way exert a 'tumour suppressor' function through its DNA binding capacity and since it is expressed in almost every normal tissue it may be implicated in tumours other than retinoblastoma [6, 7]. The link between soft tissue sarcomas and familial retinoblastoma has led various groups to examine these tumours for inactivation of RB1. Perhaps disappointingly, structural alterations of the RB1 gene (homozygous or heterozygous loss) have been found in only a small proportion of soft tissue sarcomas, although interestingly such alterations tended to occur in leiomyosarcomas [7, 8]. Therefore the inactivation of RB1 is not an essential step in the pathogenesis of all soft tissue sarcomas but may be of particular significance in leiomyosarcoma.

CHROMOSOME REARRANGEMENTS

There have been a series of reports describing primary chromosome abnormalities in soft tissue tumours, not only in sarcomas but also in benign neoplasms (e.g. a high proportion of lipomas), which are summarized in Table 1. Chromosome translocations are the most frequently observed rearrangement, and the same breakpoints have been identified consistently within particular tumour types or sub-types. Thus several reports from different laboratories have confirmed the translocation t(X;18) (p11.2;q11.2) in synovial sarcoma [9]. The interpretation of cytogenetic data is not always clear-cut, however. A specific translocation, or other chromosomal abnormality, is not necessarily seen in all tumours which have been assigned to a particular subgroup on the basis of histological classification, and it is apparent that further subgroups may be distinguished on a cytogenetic basis. For example the t(2;13) translocation is not found in all alveolar rhabdomyosarcomas and is not limited to the alveolar–rhabdomyosarcoma sub-type, and there is a suspicion now that it may represent a secondary rather than a primary abnormality [10, 11]. Similarly, subgroups of liposarcomas [12] and leiomyosarcomas [13] have been distinguished cytogenetically. The biological basis of these differences remains to be determined.

The identification of a consistent translocation breakpoint in a group of tumours indicates that this rearrangement must play a primary role in tumour development, although the finding of specific chromosome rearrangements in benign tumours indicates that such rearrangements do not necessarily fully account for malignant transformation. By analogy with the translocation breakpoints cloned from leukaemias, lymphomas, etc. (reviewed in [14]), it seems likely that the localization of chromosome breakpoints in soft tissue tumours at the cytogenetic level can direct molecular analysis to the likely positions of proto-oncogenes, and hence to genes normally involved in cell replication and differentiation. Using the reverse approach, genes which have already been mapped may be seen as candidates for involvement in specific translocations. On this point some caution must be exercized because the level of sensitivity with which genes can be localized to chromosome bands (e.g. by *in situ* hybridization) can make the apparent coincidence of cytogenetic breakpoints and oncogene localization misleading. For example, we have found no evidence for rearrangements or altered expression of the two putative proto-oncogenes A-*raf* [15] and *elk*-1 [16] in synovial sarcomas with t(X;18)

*Table 1. Primary chromosome translocations identified in soft tissue tumours**

t(2;13) (q37;q14)	rhabdomyosarcoma
t(3;12) (q27;q13–14)	lipoma
t(12;14) (q14–15;q23–24)	uterine leiomyoma
t(12;16) (q13–14;p11)	liposarcoma
t(x;18 (p11.2;q11.2)	synovial sarcoma

*Occurring as the sole anomaly in at least two neoplasms of similar morphology and reported by at least two investigators from different laboratories (for references see [9]).

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(p11.2;q11.2), even though these gene loci have been mapped to chromosomal region Xp11.2–11.4 ([17], Knight and Clark, unpublished data). Similarly, chromosomal band 12q13 is involved in rearrangements in a variety of soft tissue tumours (Table 1), but among the four genes localized to this region (the two putative oncogenes *int*-1 and *gli*, the collagen gene COL2A1 and the putative apoE receptor) no rearrangements have been found in tumours carrying the translocation [18–21]. The gene for apoE, the probable ligand of LRP (low-density lipoprotein receptor-related protein) had been a particularly strong candidate for involvement in lipoma and liposarcoma because of the proposed role of LRP in lipid metabolism. However, it is not unlikely that other genes regulating adipose tissue development are clustered in the same region [21] and could be identified through cloning the breakpoint region at 12q13. Molecular genetic analyses of chromosome rearrangements may be viewed as a means of identifying the genes controlling normal growth and differentiation in soft tissues.

Unfortunately, the mapping and cloning of translocation breakpoints in soft tissue tumours has been hampered by technical problems (the difficulty in establishing cell lines and somatic cell hybrid clones containing selected derivative chromosomes) and by the fact that such undertakings are very labour-intensive. The most significant progress has been made in mapping the t(X;18) (p11.2;q11.2) translocation in synovial sarcoma, aided by the successful isolation of somatic cell hybrid lines containing der (X) in the absence of other X and 18 material, and by the fact that a preponderance of marker probes and gene loci have already been mapped to the Xp11.2 region [22]. However, two groups have now published different conclusions about the position of the breakpoint at the molecular level based on analyses of somatic cell hybrid lines isolated independently from two tumours [17, 23]. Further tumours must be examined to resolve this discrepancy and the objective of future studies will be to home in on rearranged DNA sequences at the breakpoint junction.

The activation of proto-oncogenes by point mutation or gene amplification could also play a role in soft tissue sarcoma development. In one study mutations of N- and K-*ras* were found in 35% of rhabdomyosarcomas (5/14) [24]. Amplification of the putative oncogene *gli* has been reported in a very small number of childhood sarcomas [25, 26], and several groups have looked for overexpression of the proto-oncogene B-PDGF (*sis*), which is the ligand for receptors expressed on mesenchymal cells. *sis* was originally identified as a viral oncogene (*v-sis*) which causes sarcomas in animals and can transform fibroblasts *in vitro*. Enhanced expression of *sis* has been reported in some soft tissue tumour biopsies [27].

CONCLUSIONS AND FUTURE PROSPECTS

The evidence emerging from studies on other groups of tumours such as colon, lung and breast cancer is that cells must accumulate a series of mutations before undergoing complete transformation and this seems likely to be true also of soft tissue cells. The diversity of histological types and sub-types no doubt reflects complex patterns of genetic mutations. The studies carried out over the past few years have shown that soft tissue sarcomas carry specific genetic defects which include point mutations, allele loss and chromosome translocations. More detailed analysis of these alterations at the molecular level is necessary to understand how these events lead to malignant transformation.

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Multidrug Resistance and its Circumvention

William T. Beck

MULTIDRUG RESISTANCE (MDR) is now a common and well-studied experimental phenomenon [1–3] that appears to have clinical correlates [4] and may play a role in clinical resistance to antineoplastic agents [5]. By now, the basic features of MDR are well known. Mammalian cells selected for resistance to a 'natural product' anticancer drug display cross-resistance to a variety of other agents that have no apparent commonality, save that they are in general rather large (M_r ranging from ~300 to 900), lipophilic, and do not appear to enter cells by specific carrier proteins. Such cells accumulate and retain less drug than do their drug-sensitive counterparts, and this is presumably the cellular basis for their resistance [6]. This alteration in cellular pharmacology is likely mediated by P-glycoprotein (Pgp), a large (M_r ~ 170,000) integral membrane protein that is thought to affect the rapid efflux of drugs from the cell [1–3]. The cDNA encoding the protein has been cloned and sequenced [7–9], inserted into an expression vector, and transfected into drug-sensitive cells with the result that the transfectants express the full MDR phenotype [10, 11]. These types of experiments support the hypothesis that Pgp functions to export these large, lipophilic cytotoxic agents from the cell. Based on its normal expression in colon, small intestine, adrenal, kidney and hepatic tissues [12, 13], it has been proposed [14] that Pgp plays a role in the excretion of xenobiotics from the body.

Recent descriptions of MDR *not* associated with Pgp overexpression have added a degree of complexity to the definitions of MDR. For example, several anthracycline-resistant cell lines have been established that exhibit drug transport defects but do not appear to express Pgp [15–17]. The basis for this resistance is not clear, but it may be related to alterations in intracelllular drug distribution [15]. Furthermore, we and others have described cell lines expressing a form of MDR associated with decreases in the catalytic activity of and DNA cleavage by the essential nuclear enzyme, topoisomerase II [18–21]. Cells expressing this type of MDR, originally termed by us 'atypical' MDR (at-MDR), do not overexpress Pgp, and are unaltered in their ability to accumulate or retain drugs, compared to drug-sensitive cells. I will confine my remarks here to mechanisms of Pgp-associated MDR (Pgp-MDR).

DRUG ACCUMULATION AND RETENTION

Insights into the mechanism by which Pgp affects drug accumulation and retention come from experiments showing that a photoactive azido analog of vinblastine, a drug to which Pgp-MDR cells express resistance and cross-resistance, specifically binds to Pgp [22]. This binding can be competed to varying degrees by other 'MDR-type' drugs (vincristine, doxorubicin, etc.) as well as by 'modulator' compounds (verapamil, reserpine, etc.) [23] that can overcome this resistance by enhancing cellular levels of anticancer drug, thereby allowing more of the agent to reach its cytotoxic target(s). Despite earlier interpretations suggesting that there was little specific structural similarity among drugs or modulators associated with the Pgp-MDR phenotype [6], it now appears that these anticancer drugs and most Pgp-MDR modulators are recognized and bound by Pgp [22, 23].

MECHANISMS OF CYTOTOXICITY AND RESISTANCE

Regardless of their diverse mechanism(s) of cytotoxicity, it is clear that many 'natural product' compounds share at least one common mechanism of resistance—that mediated by Pgp. Thus, *Vinca* alkaloids (whose cytotoxicity is mediated through binding to tubulin), epipodophyllotoxins and aminoacridines (which inhibit DNA topoisomerase II activity), and anthracyclines (which appear to have membrane, cytoplasmic, and nuclear cytotoxic targets) all exhibit diminished effectiveness in cells overexpressing Pgp. More recent findings indicate that compounds that target mitochondria also share this Pgp mechanism of resistance. For example, Pgp-MDR cells are cross-resistant to the mitochondrial-specific dye, rhodamine 123 [24], as well as the quaternized bis-quinolinium phthalanilide analog, QBQ, described by Liley *et al.* [25]. The apparent mitochondrial targeting of these compounds, however, is probably irrelevant in terms of resistance mechanisms. That Pgp-MDR cells are cross-resistant to these compounds is likely due to the very real possibility that they serve as substrates for Pgp; indeed, this appears to be the case for rhodamine 123 [24, 26]. It will be of considerable interest to determine whether QBQ competes with a photoaffinity analog of vinblastine for binding to Pgp.

Pgp-MDR 'MODULATORS'

Studies of modulators of Pgp-MDR have provided some insights into both the mechanism(s) of and the direction for subsequent studies of this form of MDR. For example, recent work suggests that there are indeed structural requirements for a modulator to bind to Pgp [23, 27]. My colleagues and I proposed some 'rules' for a compound to be a good modulator of MDR—it should be hydrophobic, have two aromatic rings and a positively charged nitrogen [28]. Cationic, lipophilic molecules such as the mitochondrion-targeting agents, rhodamine 123 and QBQ, would appear to fulfill these criteria. Other agents such as cyclosporin [29], tamoxifen [30] and Tween 80 [31] also modulate Pgp-MDR, but, with the possible exception of tamoxifen, they do not appear to share all of these characteristics. These modulators, however, are all highly lipophilic, suggesting

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