Applications of Molecular Diagnosis in Soft Tissue Tumors

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ABSTRACT: This article concerns the contribution of new technological advances in the field of molecular genetics and their application for a more precise classification of soft tissue tumors. Molecular techniques offer new information about the heterogeneity and complexity of tumors, since we have at present a plethora of information related to genes and gene regulators that contribute to the complex interactions between environment and genetic background. The use of molecular diagnosis offers a more reliable classification system and can utilize even minute tissue samples. In addition, it can help improve new therapeutic ways, including gene therapy.

Key Words: Molecular diagnosis, Soft tissue tumors, Genes, Histopathology.

INTRODUCTION

Soft tissue tumors comprise a heterogeneous group of relatively rare malignancies with strong genetic predisposition and extensive diversity of histopathologic characteristics (Fletcher JA, 1996). They have traditionally been classified according to the features by which they resemble the normal mesenchymal tissues of their origin, such as cartilage, fat, skeletal or smooth muscle and nerve sheath tissue. This classification scheme however, is not very accurate in some cases either because of poor tumour cell differentiation that fails to identify any morphological similarity to a mesenchymal tissue, or due to lack of a normal counterpart (Ladanyi, 1995, Pfeifer et al., 2000).

Although immunohistochemistry can solve a number of these difficult cases, molecular analysis has significantly helped not only with the correct diagnosis of the different types of tumors, but also with their proper classification. For example the discovery of the EWS-FLI1 fusion gene derived from the t(11;22)(q24;q12) indicated that Ewing’s sarcoma and the primitive neuroectodermal tumour are related neoplasms (Delattre et al., 1994) (Table 1). Other similar cases include the dermatofibrosarcoma protubers (DFSP) and giant cell fibroblastoma (GCF) (Allen and Zwi, 1992), the congenital fibrosarcoma (CFS) and cellular mesoblastic nephroma (CMN) (Knezevich et al., 1998) and the myxoid and round cell liposarcomas (Rubin and Fletcher, 1997). A similar story applies to the alveolar rhabdomyosarcoma (ARMS) and spindle cell and embryonal rhabdomyosarcomas (Wong and Suster, 1995). Molecular analysis has also revealed that many translocations, such as those in synovial sarcoma (Fligman et al., 1995), EWS-PNET, and alveolar rhabdomyosarcoma (Edwards et al., 1997), have variation in the fusion gene partners or the exon contribution to the fusion gene (Zuckman et al., 1993). This adds a layer of complexity to the genetic classification system, but also provides important additional information.

This article provides an overview of the role of molecular diagnosis and genetic screening and its technological advances in the analysis of soft tissue tumors.
Gene types involved in tumors

Genetic alterations often tend to either increase the ability of a cell to proliferate (oncogenes), decrease the susceptibility of a cell to apoptosis (tumor suppressor genes) (Clarke et al., 1994), or promote a general state of cellular instability (Duesberg et al., 1998).

Genes that positively control the cell cycle or block apoptosis can typically be altered to become oncogenes. Types of oncogene mutations include point mutations that lead to structural alterations of protein domains and gene translocations (Sandberg, 2002). Gene translocations can either result to over-expression of an oncogene due to abnormal promoter activity (May et al., 1993), or a fused gene product, that is never seen in normal cells and is highly characteristic of the malignant disorder in which it is found (Rabbitts TH, 1998). The variability in fusion products can have clinical application in prognosis.

Tumorigenesis involves not only dominant activated oncogenes, but also recessive, loss-of-function mutations in tumor-suppressor genes. The normal functions of tumor-suppressor genes fall into categories complementary to those of proto-oncogenes. Tumor suppressor genes may be inactivated by changes at the DNA level (rearrangements, microdeletions, point mutations), but there is also increasing evidence for a third epigenetic mechanism, DNA meth-
ylation (Versteeg 17). Genome-wide disturbances of normal CpG methylation, have been demonstrated for several tumor suppressor genes in a variety of phenotypes (Jones and Laird, 1999). Unfortunately, standard techniques for mutation screening overlook changes in methylation, so their importance has probably been underestimated.

Genes that protect the integrity of the genome are the most recently recognized category of genetic alteration in human tumors. They are not directly involved in controlling the cell cycle. Instead, they have a general role as ‘caretakers’, ensuring the integrity of the genome (Kinzler and Vogelstein, 1997). Loss of function in such genes leads to a general genetic instability that has long been recognized as a feature of cancer cells. Hallmarks of chromosome instability include various gene rearrangements due to gene amplification or loss of heterozygosity (LOH), as well as abnormal telomerase activity.

**Gene amplification:** Gene amplification is defined as the accumulation of additional copies of a gene. It can be viewed as a mechanism by which cells can acquire increased expression of genes that offer a selective advantage. However, in intermediate evolutionary steps it can confer to genomic instability and lead to disease (Gisselsson et al., 1999). Although the mechanisms that underlie this phenomenon are not clear, the propensity to undergo gene amplification is clearly enhanced in malignant cells (Mengi-Sartorio et al., 2001).

**Loss of Heterozygosity:** At the molecular level, loss of heterozygosity (LOH) is associated with allelic imbalance, i.e., loss of one of the parental alleles present in the patient’s normal cells (Santarosa and Ashworth, 2004). These losses occur largely due to structural alterations that lead to chromosome misarrangement and abnormal mitotic recombination.

**Telomerase activity:** There has been much excitement over the discovery that 90% of human primary tumors possess telomerase activity. Telomerase is a repeat sequence (TTAGGG)_n of an RNA-containing enzyme system that protects the ends of chromosomes, the telomeres. Telomere length declines with time in normal somatic cells, a phenomenon known as the ‘mitotic clock’ that limits the number of divisions a cell can go through (Seger et al., 2002). Although

Telomerase is present in the human germ line, it is absent in most somatic tissues. In cancer cells, telomerase keeps being produced, an event that deregulates the «mitotic clock» and results in the immortality of these cells (Gonzalez-Suarez et al., 2000).

**Molecular diagnosis techniques**

Tumors arise by a multistep process involving a series of genetic alterations that accumulate in the cells in a fashion so characteristic of each tumor category that can be used for diagnostic purposes (Graadt et al., 1999; Pfeifer et al., 2000).

Recent techniques in chromosome analysis and molecular cytogenetics have improved our ability to characterise genetic changes in a wide panel of soft tissue tumours (Table 1). However, as with any technique, genetic assays have their advantages and disadvantages and it is important to recognize the limitations of these methods.

The most common techniques currently used in molecular diagnostics include;

**PCR amplification:** This technology enables the analysis of genetic changes in the DNA from a wide variety of tumor specimens, including cell lines, fresh or frozen tissue, and formalin-fixed paraffin-embedded material (Jackson et al., 1990). PCR is a very sensitive method that allows detection of a broad range of chromosomal abnormalities, from gross structural alterations such as translocations and deletions, to point mutations.

**RT-PCR:** Another important use of cDNA technology has allowed PCR to be applied to RNA, by generating a cDNA copy of the mRNA of interest using reverse transcriptase (Standa and Schneider, 1991). This technique is useful for the analysis of abnormal mRNAs that occur in some tumors such as specific fusion transcripts created by translocation events [e.g. the Ewing’s sarcoma (Zucman et al., 1993) and synovial sarcoma (Hiraga et al., 1998)].

**Cytogenetic analysis:** Conventional cytogenetic analysis (karyotyping) of tumor cells has proved a useful tool in molecular diagnosis. It is performed using various staining techniques that highlight chromosome bands and provides simultaneous low-resolution analysis of multiple chromosomes. It is able to identify the loss or gain of whole chromosomes,
rearrangements affecting large regions of individual chromosomes, and large deletions and amplifications (Sozzi et al., 1997). However it lacks the sensitivity to detect more subtle abnormalities such as small deletions and point mutations.

**Fluorescence in situ hybridisation:** Most molecular cytogenetic methods are currently based on fluorescence in situ hybridisation (FISH). FISH is a more versatile technique with a higher sensitivity than conventional cytogenetic analysis (Kaluzewski et al., 2003). The method concerns evaluation of particular chromosome regions using fluorescently labelled DNA probes. It can be used on a variety of tissue samples to map loci on specific chromosomes and uncover small rearrangements that are not detectable by standard karyotypic analysis (Yoshida et al., 1997).

**Chromosome painting (SKY and M-FISH):** Spectral karyotyping (SKY) (Speicher et al., 1996) and Multiplex Fluorescence In Situ Hybridization (M-FISH) (Schrock et al., 1996) are multicolour karyotyping techniques that permit the simultaneous visualization of all human chromosomes in different colors, enabling a comprehensive screen of the entire tumor cell karyotype (Liehr et al., 2004). The applications of SKY and M-FISH for screening genomes for chromosomal aberrations are manifold, by making possible the identification of complex chromosomal abnormalities, such as chromosomal breakpoints, or subtle translocations.

**MAPH vs MALP:** A significant aid in the development of molecular diagnosis have offered the two recently established amplification techniques, Multiplex Amplifiable Probe Hybridisation (MAPH) (Armour et al., 2004) and Multiplex Ligation-dependent Probe Amplification (MLPA) (Schouten et al. 2002). Both techniques are based on similar fluorescent hybridization and quantitative multiplex PCR amplification and can be used to determine relative copy number of a genomic target sequence, both at the DNA and RNA levels, as well as to detect changes at the methylation pattern of gene promoters.

**Comparative genomic hybridization:** Comparative genomic hybridization (CGH) is a fluorescent molecular cytogenetic technique that can identify genomic DNA gains, losses, and amplifications (Sijkerbuijk et al., 1997). It is based on quantitative comparison of tumor genomic DNA and normal reference DNA using two-color fluorescence in situ hybridization. The difference in fluorescence intensities along the chromosomes, are a reflection of the copy number changes of corresponding sequences in the tumor DNA. CGH has the advantage of analyzing entire genomes within a single experiment (Weber-Hall et al., 1996). It is particularly applicable to the study of tumors which do not yield sufficient metaphases for cytogenetic analysis and can be applied to a wide range of tissue preparations. It cannot however detect balanced chromosomal rearrangements, which are diagnostic markers in many soft tissue tumors and lacks the resolution required to detect small deletions.

**Representational differential analysis:** Representational differential analysis (RDA) is a recent approach with the means for isolating DNA fragments that are present in only one of two nearly identical complex genomes (Lisitsyn et al., 1995, Liang, 2002). It can detect DNA fragments amplified or deleted in cancer cells (Baldocchi and Flaherty, 1997), as well as rare transcripts (O’Neill and Sinclair, 1997). RDA can identify a simple LOH, when the deletion involves a polymorphism as well as abnormal CpG methylation that lead to silencing of tumor suppressor genes in neoplasia.

**Microarray technology:** Another approach to comparative gene expression profiling employs the use of DNA microarrays (Kurian et al., 1999). They involve the immobilization and further processing of DNA sequences in a gridded array on the surface of a solid support, such as a glass microscope slide or silicon wafer. With the development of DNA microarrays the ability to monitor the expression levels of thousands of genes simultaneously offers the potential opportunity to expand the analysis of cancer genetics beyond single-candidate gene approaches, toward considering genetic networks (Hahn and Weinberg, 2002).

**CONCLUSIONS**

Statistical analysis has indicated that more than 1% of all human genes are involved in the development of tumors. The expansion of knowledge in the field
Εφαρμογές της μοριακής διάγνωσης στους όγκους μαλακών μορίων

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ΠΕΡΙΛΗΨΗ: Το άρθρο αυτό παρουσιάζει επισκοπικά τη συμβολή της νέας τεχνολογίας/μοριακής διάγνωσης στην ακριβεστερή ταξινόμηση των νεοπλασμάτων των μαλακών μορίων. Η εφαρμογή της μοριακής γενετικής στη διαφορική διάγνωση προσφέρει πολλές νέες πληροφορίες σχετικά με την ετερογένεια και πολυπλοκότητα διαφόρων νεοπλασματικών νόσων. Ο ρόλος πολλών γονιδιακών ρυθμιστών καθώς και τροποικίων γονιδίων που συμβάλλουν στην πολυπλοκότητα της σχέσης μεταξύ γονότυπου και περιβάλλοντος, έχει διερευνηθεί και κατανοηθεί. Η χρήση της μοριακής διάγνωσης θα προσφέρει ένα περισσότερο αξιόπιστο σύστημα ταξινόμησης νεοπλασμάτων, διευκολύνει την ανακάλυψη δεικτών πρόγνωσης για την ανύλητη απειροελάχιστων δειγμάτων της νόσου και έχει τη δυνατότητα ανάπτυξης νέων θεραπευτικών οδών, συμπεριλαμβανομένης και της γονιδιακής θεραπείας.

Λέξεις Κλειδιά: Μοριακή διάγνωση, Ιστοπαθολογία, Όγκοι μαλακών μορίων, Γονίδια.

REFERENCES


