Review

Molecular Tools Used in the Study of Sarcomas

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Definition and General Characteristics of Sarcomas

Sarcomas are malignant tumors that can develop in the connective tissue throughout the body and may present under different histological types. The name sarcoma has Greek origin and means *growth of meat or meaty growth*.[1] In early stages, patients do not present symptoms since soft tissues are relatively elastic and enable tumor growth without presenting alterations or sequelae.[2] These neoplasms are relatively rare; however, affected patients have high rates of mortality and morbidity.[2-4]

Sarcomas are histologically classified according to the tissue in which it is differentiated; for example, a malignant tumor with adipocytic differentiation is called liposarcoma and a tumor with smooth muscle differentiation is called leiomyosarcoma.[1,2,5]

The diagnosis of these tumors is difficult and is based on the histological type and degree of the tumor.[6] Treatment is performed in accordance with the extension of the disease (tumor size, degree of malignancy and dissemination) and treatment options include surgery, chemotherapy and radiotherapy.[2,3,7]

Gene Expression Analysis of Sarcomas

Sarcomas can present histologically similar anatomy, topographical location and appearance.[3,8] These factors, as previously stated, make the precise diagnosis of these tumors difficult. Additionally, there are some situations in which the immunohistochemical panels used in routine laboratory diagnosis are not sufficient to finalize the diagnosis. In these cases, the pathologist depends on the help of molecular techniques, such as fluorescent *in situ* hybridization (FISH) that permits the detection of specific translocations. On the other hand, knowledge of genetic and molecular alterations can also help in the choice of treatment to be employed.

In recent decades, numerous specific genetic abnormalities have been described that serve as a diagnostic tool for many cases of sarcomas. The principal genetic alterations found in the different tumor groups are mutations, deletions or amplifications and translocations.[9,10]

Some histological types of sarcomas possess specific genetic characteristics; for example, the chromosome translocations observed in synovial sarcomas t(x;18), dermatofibrosarcoma protuberans t(17;22), and alveolar sarcomas t(2;13), among others.[5,9,10] However, there are sarcomas that do not have specific alterations, presenting complex disorganization of their karyotype and an elevated chromosomal instability.[10,12] In this context, the overall analysis of gene transcripts has been increasingly useful in the description of the taxonomy, diagnosis and in the treatment choice of different types of tumors. The knowledge of the gene expression profile will enable a greater understanding of sarcomas and certainly will help in the diagnosis and prognosis of different histological types.

In recent years, many studies have presented promising results regarding the identification of molecular gene markers. These genes present a unique expression

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profile of each histological type of tumor, or in other words, a type of "molecular signature." Thus, differentially expressed genes, cytologic changes and chromosome changes unique to each histological type of sarcoma have been identified. [9,11]

In the literature, a series of examples can be found of the usefulness of gene expression analysis through modern molecular biology techniques. A good example is the synovial sarcoma which presents diagnostic difficulty, as it is histologically similar to other fusiform tumors (e. g, fibrosarcomas and rhabdomyosarcomas, among others). [2,5,8] Recently, studies have shown the elevated expression of genes involved in growth, adhesion and cellular cycle (ERBB2, IGF2 and IGFBP2, respectively) in synovial sarcomas. [11,12] In addition, genes involved in the tumor growth factor beta (TGFβ) signaling pathways (RVNZ3, SMAD6, TGFβ1 and TGFβ2) and Frizzled-Wnt (WNT5, FZD1, TLE-1, MEOX 2, EPHB3 and SALL) were also found with greater transcript quantity in these tumors. [12] In this last group of genes, the most interesting results consider the expression of Transducin–like enhancer of split 1 (TLE-1) that is able to distinguish synovial sarcomas from all other sarcomas, for both the quantity of transcript [13] and protein expression. [5,13-16] Currently, the tle-1 protein assists in the diagnosis of synovial sarcomas. This discovery was of great importance, since the synovial sarcoma can be confused with various tumors and their diagnosis of certainty can only be done through translocation t (x;18) by FISH method [17] or RT-PCR techniques that are not accessible to all routine laboratories.

Molecular Techniques Used in the Study and Diagnosis of Sarcomas

The development of new technologies made the realization of molecular biology possible in routine clinical laboratories, and in addition, much of the research developed currently uses modern techniques of analysis. The most utilized techniques in the study of neoplasms are complementary DNA (cDNA) microarray, quantitative real time PCR (QRT-PCR), comparative genomic hybridization (CGH), fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH), silver in situ hybridization (SISH), and the sequencing of DNA. [9,10]

Each of these techniques has its advantages and limitations; for example, cDNA microarray serves as an initial screening and allows the identification of genes that present differences in the expression profile. [18] With this technique it is possible to know, for example, which genes are differentially expressed among the different types of sarcomas. However, the results obtained with this technique need experimental validation, in other words, it is necessary that another technique reproduce the same result. At present, QRT-PCR is an extremely sensitive and independent technique and the obtained results are trustworthy and specific. [19] The restriction for using QRT-PCR is to have quality RNAs and similar profiles for all the desired study samples.

Both cDNA microarray and QRT-PCR are being widely used for the study of sarcomas in general. According to the indexed digital database PubMed, approximately 650 works have been published to date using these two techniques for the analysis of gene expression in these tumors. These studies are extremely important since sarcomas are rare tumors and little is known about their origin and biological behavior.

CISH/SISH and FISH are techniques that are based on the same principle, using probes (DNA fragments) that bind by a target sequence homology. [20,21] The detection in FISH occurs by capturing the fluorescence of the probe by using a specific microscope for analysis. In CISH/SISH, detection occurs through a peroxidase system (or alkaline phosphatase) using the chromogen DAB (3,3'-Diaminobenzidine) [22] and staining by silver deposition, [23] respectively. These techniques are based on the complementarity of DNA chains and allow, among other things, the detection of gene amplification or deletion, chromosomal translocation, alteration in the number of chromosomes, and chromosomal location of a given gene. [20-23] Currently, there are some technical variations, such as RxFISH and M-FISH, which are multicolored. These technologies are more recent and are based on a combination of five or more fluorophores to obtain a specific color banding pattern.

FISH is still widely used in cases of diagnostic doubt in synovial sarcomas, as stated earlier, and in the study of specific translocations for various types of sarcomas. In addition, FISH has been widely used in the studies of the deletion of tumor gene suppressors and the amplification of genes involved in the development of sarcomas.

Another technique used in the study of sarcomas is the sequencing of DNA. [24] This technique enables the analysis of genes of interest, allowing the detection of mutations, deletions, polymorphisms, methylation and translocation, among other genetic and epigenetic events. [24,25] This technique is very sensitive and specific; its limitation is the need for prior knowledge of the

nucleotide sequence of the gene being examined. The majority of published studies using sequencing in sarcomas present the analyses of mutations, deletions and translocations of the genes investigated or related to some other type of cancer.

Starting from the 1990s, the technique of comparative genomic hybridization (CGH) has been applied in sarcoma research. This is a cytogenetic-molecular method that allows the detection of loss, gain and amplification of the number of gene copies at the chromosomal level. [26] The technique is based on the hybridization of tumor DNA marked with fluorescence, generally FITC, and normal DNA also marked with rhodamine or Texas Red. The limitation of this technique is the ability to detect only chromosomal changes. [26-28]

Generally, we can say that the advances of molecular techniques have improved diagnostic possibilities and have allowed a major advancement in knowledge of various diseases. We know that several factors exist that hamper the study of sarcomas, being that they are rare tumors whose study is possible only in reference centers, and not knowing the cell which gives origin to each of its histological subtypes. Accordingly, most of the knowledge we have today on behavior, diagnosis, prognosis and treatment of these tumors is due to studies conducted with the aid of molecular tools.

References

1. Cotran RS, Kumar V & Collins T. 1999. Robbins pathologic basis of disease. 6º ed. Ed. Saunders Company Editora. USA.
2. Gill PG (trad. Figueiredo MTA). 1999. Sarcoma de partes moles. In: Manual de Oncologia Clinica. 6º ed. Ed. Springer-Verlag UICC. Fundação Oncocentro de São Paulo. Brasil.
3. Iwamoto Y. 1999. Diagnosis and treatment of soft tissue tumors. J Orthop Sci. 4(1):54-65. Review.
4. Karakousis CP, Emrich LJ, Rao U & Khalil M. 1988. Selective combination of modalities in soft tissue sarcomas: Limb salvage and survival. Sem Surg Oncol. 4: 78-81.
5. Thway K. 2009. Pathology of soft tissue sarcomas. Clin Oncol (R Coll Radiol). 21(9):695-705.
6. Begnami MDFS & Soares FA. 2003. O diagnóstico do câncer no laboratório de patologia. In: Bases da Oncologia. Editores: Brentani MM, Coelho FRG & Kawdski LP. 2º Ed. Editora Maria e Tec Med. São Paulo. Brasil.
7. Abbas JS, Holyoke ED & Karakousis CP. 1981. The surgical treatment and outcome of soft tissue sarcoma. Arch Surg. 116:765-769.
8. Angervall L & Kindblom LG. 1993. Principles for pathologic-anatomic diagnosis and classification of soft-tissue sarcomas. Clin Orthop Relat Res. 289:9-18. Review.
9. Skubitz KM & D'Adamo DR. 2007. Sarcoma. Mayo Clin Proc. 82(11):1409-32.
10. Binh MBN. 2006. Soft tissue sarcomas: Update on molecular data. Cancer/radiothérpie. 10: 15-21.
11. Tschoep K, Kohlmann A, Schlemmer M, Haferlach T & Issels RD. 2007. Gene expression profiling in sarcomas. Crit Rev Oncol Hematol. 63(2):111-24.
12. Allander SV, Illei PB, Chen Y, Antonescu CR, Bittner M, Ladanyi M & Meltzer PS. 2002. Expression Profiling of Synovial Sarcoma by cDNA Microarrays. Am Journal of Pathol. 161:1587-1595.
13. Cunha IW. 2009. Genes diferencialmente expressos em tumores de origem mesenquimal. Tese de Doutorado. Fundação Antonio Prudente.
14. Nielsen T, Rubin B & Ruttan C. 2005. Expression of Groucho/Transducin-like enhancer of split protein distinguishes synovial sarcoma from malignant peripheral nerve sheath tumor. Connective Tissue Oncology Society Web site.
15. Terry J, Saito T, Subramanian S, Ruttan C, Antonescu CR, Goldblum JR, Downs-Kelly E, Corless CL, Rubin BP, van de Rijn M, Ladanyi M & Nielsen TO. 2007. TLE1 as a diagnostic immunohistochemical marker for synovial sarcoma emerging from gene expression profiling studies. Am J Surg Pathol. 31(2):240-6.
16. Jagdis A, Rubin BP, Tubbs RR, Pacheco M & Nielsen TO. 2009. Prospective evaluation of TLE1 as a diagnostic immunohistochemical marker in synovial sarcoma. Am J Surg Pathol. 33(12):1743-51.
17. Terry J, Barry TS, Horsman DE, Hsu FD, Gown AM, Huntsman DG & Nielsen TO. 2005. Fluorescence in situ hybridization for the detection of t(X;18)(p11.2;q11.2) in a synovial sarcoma tissue microarray using a breakapart-style probe. Diagn Mol Pathol. 14(2):77-82.
18. Voduc D, Kenney C, Nielsen TO. 2008. Tissue microarrays in clinical oncology. Semin Radiat Oncol. 18(2):89-97.
19. Valasek MA & Repa JJ. 2005. The power of real-time PCR. Adv Physiol Educ. 29: 151–159.
20. Trask B. Fluorescent in situ hybridization. 1998. In: Genome Analysis: A Laboratory Manual. (Vol 4). Editors: Birren B, Green ED, Hieter P, Klapholz S, Myers RM, Riethman H, and Roskams J. Plainview, NY. Ed. Cold Spring Harbor Laboratory Press. Pg. 303.
21. Blankato JK. Fluorescent in situ hybridization. 1999. In: The Principles of Clinical Cytogenetics. Editors: Steven L. Gersen and Martha B. Keagle. Totowa, NJ. Ed. Humana Press Inc. Pg. 443.
22. Zhao J, Wu R, Au A, Marquez A & Yu Y and Shi Z. 2002. Determination of HER2 Gene Amplification by Chromogenic In Situ Hybridization (CISH). Mod Pathol. 15(6):657–665.
23. Dietel M, Ellis IO, Höfler H, Kreipe H, Moch H, Dankof A, Kölble K & Kristiansen G. 2007. Comparison of automated silver enhanced in situ hybridisation (SISH) and fluorescence ISH (FISH) for the validation of HER2 gene status in breast carcinoma according to the guidelines of the American Society of Clinical Oncology and the College of American Pathologists. Virchows Arch. 451:19–25.
24. Sanger F, Nicklen S & Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors (DNA polymerase/nucleotide sequences/bacteriophage 4X174). Proc Nati Acad Sci. 74 (12): 5463-5467.
25. Castric V, Bechsgaard JS, Grenier S, Noureddine R, Schierup MH & Vekemans X. 2010. Molecular Evolution within and between Self-Incompatibility Specificities. Mol Biol and Evol. 27(1):11-20.
26. Snijders AM, Nowak N, Segraves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hindle AK, Huey B, Kimura K, Law S, Myambo K, Palmer J, Ylstra B, Yue JP, Gray JW, Jain AN, Pinkel D & Albertson DG. Assembly of microarrays for genome-wide measurement of DNA copy number by CGH. Nat Genetics. 29: 263-264.
27. Tirkkonen M, Tanner M, Karhu R, Kallioniemi A, Isola J & Kallioniemi OP. 1998. Molecular Cytogenetics of Primary Breast Cancer by CGH. Genes, Chromosomes & Cancer. 21:177–184.
28. Micci F, Teixeira MR, Bjerkehagen B, Heim S. 2002. Characterization of supernumerary rings and giant marker chromosomes in well-differentiated lipomatous tumors by a combination of G-banding, CGH, M-FISH, and chromosome-and locus-specific FISH. Cytogenet Genome. 97(1-2):13-9.

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