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Molecular biology of cutaneous T-cell lymphomas

E. Berti
S. Gimelli+
L. Venegoni
V. Merlo
R. Riboni*
O. Zuffardi+
M. Paulli*

*Institute of Dermatological Science, IRCCS-Fondazione Ospedale Maggiore, Mangiagalli and Regina Elena and university of Milan, Milan-Bicocca; *Anatomic Pathology and +Genetics, IRCCS-Ospedale S.Matteo, University of Pavia, Italy*

Actually, the diagnostic procedures for the molecular diagnosis and monitoring of a T-cell neoplasia on tissue sections or DNA extracted from cutaneous lesions include:

a. the demonstration of clonality (a clone is a group of cells expanded from a single cell), of the lymphoid tissue infiltrates, by using molecular probes specific for the T-cell receptors (TCR); TCR-Gamma, TCR-Beta and, more rarely TCR-Delta subunits. This analysis, in the past done by using Southern-Blot method, may be performed by using polymerase chain reaction (PCR) non-radioactive very sensitive methods, such as heteroduplex analysis of TCR[1] on DNA extracted from lesional skin and may be indicative of malignancy, because reactive cutaneous lymphoid infiltrates are usually polyclonal.

b. the detection of chromosomal translocations by Southern-Blot, PCR on the extracted DNA or on tissue sections/cytological smears by the new fluorescence *in situ* hybridization (FISH) techniques : i.e. the t (2;5) translocation detected in the systemic CD30+ anaplastic large cell (ALC) lymphomas and not in primary cutaneous CD30+ LC lymphomas or lymphomatoid papulosis (LYP).[2]

c. the demonstration of oncogenes or oncosuppressor genes involvement, by PCR method or FISH, mainly useful to the understanding of the pathogenesis and the progression/transformation processes characteristics of these diseases: such as p16, p53, lyt-10, tal-1.[3]

d. the involvement of specific viruses (by quantitative PCR or FISH), such as HTLV1 and EBV, but also HHV6, HHV7, HHV8.

e. the demonstration of chromosomal imbalances on DNA extracted from the cutaneous lesion by using the array-CGH (comparative genomic hybridization): this is a technique for the comparative hybridization of the genome based on array; platforms in which genomic clones containing specific sequences of the human genome (genic and extragenic sequences) are located, the clones are distributed at different distances one from the other, depending from the type of array used.

Traditional cytogenetic has a resolution of 10Mb, high resolution HR-CGH on metaphases has a resolution of 5 Mb, whereas array-GH has a resolution of 100Kb and now of 6Kb.[4]

f. the determination of the gene expression profile (GEP) of the infiltrate neoplastic cells on the tissue extracted RNA and cDNA, to look for marker genes (always low or highly expressed) to use for diagnosis, could be in imbalanced areas of the genome. Recently a group of 27 genes had been defined for the diagnosis of mycosis fungoides.

In our study we analyzed by a very sensitive oligo-array. CGH methods the DNA extracted from the cutaneous lesions of a group of rare CTCL classified by the recent WHO/EORTC classification.[5]

Materials

a) NK-blastic/haematodermic neoplasia HN CD4+ CD56+ (9 cases); b) CD8+ lymphomas, aggressive and non aggressive (7 cases, 8 cases); c) NK/T cells extranodal nasal-type lymphoma (4 cases); d) subcutaneous panniculitis like CD8+ alpha-beta T-cell lymphoma (3 cases); gamma-delta lymphoma (2 cases); primary cutaneous lymphoblastic lymphoma (2 cases).

Methods

Array-CGH was performed using Agilent Human Genome CGH Microarray Kit 44B.This platform is a high resolution 60-mer oligonucleotide-based

microarray that allows genome-wide survey and molecular profiling of genomic aberrations with a resolution of 75Kb. Labeling and hybridization were performed following the protocols provided by Agilent. Briefly, 4 ug of purified DNA of the patient and of a female control (Promega) were double digested with RSAI and ALUI for two hours at 37C°. After columns purification 1ug of each digested sample was labeled by random priming (Invitrogen) for two hours using Gy5-dUTP for the patient DNA and Cy3-dUTP for the control DNA. Labeled products were columns purified and prepared accordingly to the Agilent protocol. After probes denaturation and preannealing with 50ug of Cot-1 DNA, hybridization was performed at 65C° with rotation for 40 hours. After two washing steps the array was analyzed with the Agilent scanner and the Feature Extraction software (v8.0). Grafical overview was obtained using the CGH analytics software.

Results

Multiple chromosomal abnormalities including deletions, duplication, monosomies, trisomies were detected in most cases.

a) HN-CD4$^+$CD56$^+$ lymphomas: multiple chromosomal imbalances were detected in 7 out of 9 cases. The abnormalities involved several chromosomes: case 1 (chromosomes n°3, 10, 12, 13), case 2 (n°7, 9, 12, 13, 14, 16); case 3 (n°5, 6, 8, 9, 11, 12, 13, 16, 17, 18, 22, Y); case 4 (n°3, 6, 9, 12, 13, 19, X); case 5 (n°1-21, X, Y); case 6 (n°9, 13).

b) CD8$^+$ aggressive lymphomas: multiple imbalances were found in 7 cases; case 1 (chromosomes n°1, 2, 5, 6, 7, 8, 11, 15, 17); case 2 (n°4, 6, 7, 8, 9, 11, 12, 16, 18, 19, 22); case 3 (n°1, 6, 7, 9, 12, 16); case 4 (n°1, 2, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16, 17, 19, 20, 22, Y); case 5 (n°1, 2, 4, 14, 16, 17, 19, 20, X); case 6 (n°1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 18, 21, X);case 7 (n°1, 4, 7, 8, 9, 13, 17, 18, 20).

c) CD8$^+$, CD30$^+$ ALC lymphomas: multiple abnormalities were detected in 2 cases, whereas abnormalities were found in 1 out of 3 LYP cases: ALC case1 (chromosomes n°1, 9, 11, 19, X); ALC case 2 (n°1, 3, Y), LYP case1 (n°1, 4, 5, 8, 11, 16, 18, 19, 22).

d) CD8$^+$ mycosis fungoides: no abnormalities in the 3 cases analyzed.

e) CD8$^+$ alpha-beta+ SPTCL: no abnormalities in 3 cases.

f) Gamma-delta cutaneous lymphomas: abnormalities were detected in 1 out of 2 cases; case 1 with subcutaneous involvement (chromosomes n°3, 4, 9).

g) NK/T EBV$^+$ lymphomas: multiple imbalances were detected in all cases; case 1 (chromosomes n°1, 5, 6, 7, 8, 19, X); case 2 (n°1, 13, 17); case 3 (n°1, 7, 8, 9, 15, 17, 18, 19, 20); case 4 (n°8, 9, 12, 16, 17, 18, 20, X).

h) Primary cutaneous lymphoblastic lymphomas: abnormalities were detected in the 2 cases: case 1 pre-B LBL (chromosomes n°4, 6, 9, 18, 21, X), case 2 T-LBL (n°1, 4, 8, 11, 16, 19).

Discussion

Molecular biology give us several opportunities to better define and classify cutaneous lymphomas. The demonstration of clonality, sequences and translocation are useful tools for the diagnosis and monitoring of the disease. The involvement of oncogenes or oncosuppressor genes are relevant for the pathogenesis of these disorders, however the new data coming from cytogenetics analysis by using oligo-array-CGH and array-GEP techniques may be able in the next years to complitely define most of the chromosomal abnormalities/imbalances and the gene profile of these disorders.

In our studies we clearly demonstrated that, by using oligo-array CGH on DNA extracted from lesional skin, we can detect several chromosomal abnormalities involving multiple genes. Several of these abnormalities are present in most cases of the same entity, and in the same entity the case that have had indolent course show frequently less or no abnormalities. In fact, only one of the patients affected from HNCD4+CD56+ lymphoma is living and present a normal CGH profile. Moreover in seven very aggressive CD8+ lymphomas (5 primary and 2 secondary to MF) multiple chromosomal abnormalities were detected confirming their differences with CD8+ positive MF in which no chromosomal anomalies were found. All the cases analyzed of NK/T extranodal nasal type lymphomas showed multiple abnormalities involving several chromosomes; and the same was for the 2 LBL analyzed. However several abnormalities were also detected in cases of CD30$^+$ CD8$^+$ primary cutaneous ALC and in 1 case of LYP showing a favorable evolution with spontaneous regression or indolent corse. So, only the complete analysis, the confirmation of the data obtained by PCR and the comparison of the data obtained with those reported in the literature from other groups, in the same disorders and in other neoplasias, could be used for classification, prognostic evaluation and indication of the more appropriate therapy.

References

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