Editoriais/Editorials

Molecular-cytogenetic variant in chronic myeloid leukemia

Variantes citogenético-moleculares na leucemia mielóide crônica

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Chronic myeloid leukemia (CML) was the first neoplastic disease to be linked to a genetic abnormality and is the best studied molecular model of leukemia. The increased and cumulative knowledge of this disease has recently received special attention due mainly to the new therapeutic option, a signal transducer inhibitor, the imatinib mesylate.

CML diagnosis is based on the presence of the translocation between chromosomes 9 and 22, known as the Philadelphia chromosome (Ph), which provides the physical link between BCR and ABL genes.

At diagnosis, the karyotype is still the first test to be made to detect the Ph chromosome, though, besides the t(9;22), it may reveal other concomitant translocations, which may or may not indicate clonal evolution and consequent accelerated/blastic phase. The so-called "Philadelphia variant" (Phvar), which involves different chromosomes besides 9 and 22, may also be detected by this method. Phvar has been found in 5% of CML cases.[1]

At diagnosis, fluorescent in situ hybridization (FISH) and reverse-transcriptase polymerase chain reaction (RT-PCR) are extremely useful for cases in which the karyotype does not show abnormalities (masked Ph),[2] or the marrow fibrosis eludes adequate sample collection for chromosomal analysis. As molecular methods do not allow the observation of concomitant cytogenetic abnormalities, they have been performed in addition to karyotyping.

FISH is a practical technique for demonstrating the location of specific nucleic acid sequences (probe) in individual metaphase or interphase cells. These DNA probes contain a label (fluorescence) that allows their detection after hybridization to the target of interest.[3]

Using FISH probes, very interesting data about CML have been demonstrated. The first generation of FISH probes (single fusion) was manufactured in order to show a signal for ABL gene (for example, a red dot – digoxigenin) and a signal in a different color for BCR gene (for example, a green dot – FITC). In a normal cell four separated colored signals are seen, two red and two green, while in an abnormal cell with Ph, three colored dots, an isolated red, an isolated green and a fusion signal, red/green or yellow dot, corresponding to the BCR/ABL rearrangement, are observed.

These single fusion probes can be used to rapidly diagnose, in interphase or metaphase, cells with t(9;22) if the cells comprise the majority of the sample.[4] However, as there may be, due to random chance, a mere geographic juxtaposition of red and green signals in the cell imitating a false fusion, even normal samples may have a background false-positive. So, each laboratory has to establish its normal range through the analysis of control samples from healthy individuals. This value varies from 3% to 15% according to technical skills in each lab.[5]

The second set of FISH probes was manufactured in order to bypass this inconvenient false-positivity. It was designed to exhibit a small extra signal (ES) (red, for instance) on the derivative chromosome 9 by making the ABL probe larger so that a residual signal is left on the 9q. Thus, the normal pattern would still be two isolated

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red dots and two isolated green dots similar to what is seen through the single probe. But in the abnormal cell there would be a green signal, two red signals, one corresponding to the normal allele, one smaller (corresponding to the derivative 9q) and a yellow signal corresponding to the BCR/ABL fusion. In this way, only the cells with the extra signal would be counted as bearing the Ph translocation. The level of detection is increased and the normal range is more accurate.[4,6]

Another set of FISH probes, known as dual fusion set (DF) was designed to span the break-points, leaving behind a residual signal on the derivative 9q proximally and extending the signal on the derivative 22q below the break. In other words, the probes hybridizing to ABL and to BCR are larger and this creates a second fusion signal on the derivative 9q. So, with this probe set, normal cells would still be detected as two isolated green and two isolated red signals, while the abnormal cell would show two fusion signals, one smaller, and a red and a green signal. The advantage of this combination is that the resulting abnormal pattern would occur very rarely as false positive or artifact. The sensitivity of the dual fusion set is very good depending on how many cells are counted.[7]

Due to the increasing use of these probes, a number of cases that present a variety of abnormal situations, like the loss of the extra signal or of the dual fusion in the presence of Philadelphia, are being detected. These situations are also being called variants, but more specifically, "molecular-cytogenetic variants".[8] Cases with deletion of BCR or ABL genes near the regions where those enlarged probes hybridize show that additional phenomena to the translocation occur and are perhaps related to a worse prognosis.[9-11]

The RT-PCR is also a powerful method for the detection of specific sequences and allows the detection of different transcripts giving rise to the 210 kDa BCR/ABL protein, most frequent in CML (M-bcr), the 190kDa BCR/ABL protein, frequent in Ph-positive acute lymphoblastic leukemia (ALL) (m-bcr), or the 230kDa BCR/ABL protein, very rarely found (μ-bcr).

Some studies have shown no correlation between CML chronicity and breakpoint sites while others have shown that m-bcr breakpoints develop blast crisis with monocytosis.[12]

The case presented in this issue by Carvalho et al, is a clear example of a usual clinical CML presentation that hides diverse molecular aspects, proving that this disease has a multitude of intriguing underlying biological events. Cases like this can only be detected by performing conventional chromosomal G-banding, FISH and RT-PCR altogether.

CML deserves more investigational studies directed to elucidate these aspects and still has a lot to teach us.

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Avaliação: O tema abordado foi sugerido e avaliado pelo editor.
Conflito de interesse: não declarado

Recebido: 20/08/2003
Aceito: 25/08/2003