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Letter to the editor

Detection of cytogenetic abnormalities in mature B-cell neoplasms: the value of cultures with different mitogens



Mature B-cell neoplasms are a common group of hematological malignancies in Western countries with the most prevalent being chronic lymphocytic leukemia (CLL). Clonal cytogenetic abnormalities are observed in only 30% of the CLL cases studied by conventional cytogenetic techniques (karyotyping) and up to 80% of the cases studied using the fluorescence in situ hybridization (FISH) technique with probes to investigate 6q21,11q22-23 (ATM), 13q14.3 and 17p13 (P53) deletions and for trisomy 12. Cytogenetic abnormalities are associated with prognosis; deletion of 13q is associated with the best prognosis and deletion of 17p with the worst. Despite the greater sensitivity of FISH, it is expensive and does not detect abnormalities other than those investigated. Conventional cytogenetic studies in CLLs are often disappointing because no metaphases or only normal metaphases are obtained.

It is very important to send a sample containing the neoplastic cells to be analyzed to the lab for the cytogenetic study. In lymphoid malignancies this sample can be from a lymph node, the spleen, or other affected tissue, including bone marrow and peripheral blood when leukemized. The use of mitogenic agents to stimulate B-cells in cultures has been useful. The potent tumor promoter 12-myristate 13-acetate (TPA) has given the highest clone detection rate in the cytogenetic laboratory. Moreover, culturing is performed without any other stimulating agent. In the late 1990s, the use of new cytogenetic protocols with different mitogens such as the CpG-oligonucleotide DSP30 plus interleukin-2 (IL-2) improved the detection of clonal abnormalities in up to 80% in CLL. DSP30 also induces the IL-2 alpha receptor (possibly via CD25) in CLL cells thereby allowing IL-2 to generate a co-stimulatory effect.

The combination of these different mitogens was described by three different groups.

A study by Dicker et al. in Germany, with 132 CLL samples, showed successful cultures in 125 cases, 81% of which showed clonal abnormalities with the most prevalent being del6q, del11q, +12, del13q, del17p and the 14q32 rearrangement.¹

In a Brazilian study conducted by Morato et al., clonal cytogenetic abnormalities were detected in 80% of the 35 cases studied, showing recurring abnormalities (del6q, del11q, +12, del13q, -17/del17q, 14q32 rearrangement) as well as many others (+4, +5, +8, +11, +15, del12p13, +18, +19, +21, and balanced translocations involving different chromosomes).²

A study by Wren et al. in Australia failed to reproduce these data. Forty-five samples of CLL were submitted to cultures stimulated with TPA, DSP30 together with IL-2 and combined TPA and DSP30+IL-2. The rate of detection of chromosomal abnormalities was higher, significance, in the cultures with TPA+DSP30+IL-2 (52.9%) than with TPA (45.8%) or DSP30+IL-2 (29.2%). Additionally, there was a greater success in the culture with TPA (100%) than with DSP30+IL-2 (72.7%) and the mitotic index and resolution of bands were also more successful in the TPA culture.³

In view of these conflicting data in the literature, we compared the detection of clonal abnormalities using three different cultures mentioned in the literature: without stimulating agent, TPA and DSP30+IL-2 (200 U/mL) and another with a higher concentration of IL-2 (DSP30+IL-2 [400 U/mL]) using 16 samples of mature B-cell neoplasms (11 were CLL). At least ten metaphases from each culture were analyzed in an Ikaros karyotyping System (MetaSystems) by two analysts, and the karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013. Table 1 shows the results in cases with clonal abnormalities.

The incidence of clonal abnormalities in mature B-cell neoplasms in this study was 50%, with trisomy 12 being the most common abnormality. The culture with DSP30+IL-2 (400 U/mL) showed a lower failure rate than that with DSP30+IL-2 (200 U/mL). There was no detection of new clonal abnormalities in the DSP30+IL-2 culture compared to TPA. The culture with the oligonucleotide increased reagent costs of karyotyping by 3% with a lower resolution in G-banding; however, it was able to detect a higher percentage of cells with aberrant clones. Perhaps the combination of the three

Table 1 – Cytogenetic abnormalities and percentage of abnormal clones in cultures without stimulant, with TPA, DSP30 together with IL-2 (200 U/mL) and DSP30 together with IL-2 (400 U/mL).

Cytogenetic abnormalities/neoplasms	Culture without stimulant (24 and 48 h) (%)	Culture with TPA (72 h) (%)	Culture with DSP30 and IL-2 (200 U/mL) (72 h) (%)	Culture with DSP30 and IL-2 (400 U/mL) (72 h) (%)
-20 (CLL)	Absence of metaphases	20.0	Absence of metaphases	-
+12 (CLL)	0	60.0	Absence of metaphases	-
+12, del(14)(q22q24) (B cell neoplasms)	83.3	85.7	Absence of metaphases	-
XXY, der(1)t(1;?)(p13;?), der(3)t(3;?)(p13;?), del(6)(q21)x2, -8, -9, -10, +13, add(19)(p13.3), -22 (B cell neoplasms)	30.0	20.0	-	80.0
+12/del(20)(q11.2) (CLL)	50.0/0	55.5/22.2	-	80.0/0
t(1;13)(q?25;q14), add(17)(p12), -18 (CLL)	10.0	30.0	-	60.0
i(17)(q10) (CLL)	0	20.0	-	50.0
+1, del(1)(p34) (B cell neoplasms)	Absence of metaphases	10.0	-	40.0

TPA: 12-myristate 13-acetate; oligonucleotide: DSP30; IL-2: interleukin-2.

mitogens may be an effective approach to karyotype mature B-cell neoplasms as suggested by the Australian group.

Conflicts of interest

The authors declare no conflicts of interest.

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