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Sensitivity of an immunoglobulin heavy chain gene polymerase chain reaction primer system for routine diagnosis of lymphomas

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Aims

Polymerase chain reaction (PCR) and length fragment analyses of the immunoglobulin heavy chain (IgH) gene are useful tools for clonality assessment in malignant B-cell-lymphomas and reactive lymphoid infiltrates. The present investigation analyzes the sensitivity of an IgH gene consensus primer multiplex PCR system for the detection of clonality for routine diagnosis in 109 lymphomas during a period of 3 years (2003–2005) at the Institut of Pathology Klinikum Darmstadt.

Methods

We used FR2A/JH/VLJH and FR3A/JH/VLJH primer sets for detecting clonal B cell populations. Primer sequences used for PCR: Variable region FR2A consensus primer: FR2A: TGG(AG)TCCG(AC)CAG(GC)C(CT)(CT)C(AGCT)GG. Joining region (JH) consensus primer: LJH: TGAGGA GACGGTGACC, VLJH: GTGCAGGT(AGCT) CCTTGGCCCCAG-FAM. 1. PCR: FR2A/LJH and FR3A/LJH. 2. PCR: FR2A/VLJH-FAM and

FR3A/VLJH-FAM. Fluorescence fragment analyses of IgH gene rearrangement were performed with FAM-labeled PCR products by high-resolution capillary electrophoresis using the ABI-PRISM 310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany) with a POP 6-filled capillary (Applied Biosystems) and analyzed by using the GeneScan software (Applied Biosystems). A fragment was considered to be clonal in the case of a peak-height ratio (PHR) >2. The PHR was calculated as the quotient of the highest peak divided by the mean height of the two peaks surrounding the largest peak.

Results

See Table 1.

Conclusion

The present study showed a variable sensitivity of PCR of IgH gene region with FR2A/JH/VLJH and FR3A/JH/VLJH consensus primer sets for different lymphomas. Sensitivity of our consensus primer set mainly depends on the

Table 1:

Lymphoma diagnosis	Detection of clonally rearranged IgH gene
B lymphoblastic lymphoma	100% (1/1)
Mantle cell lymphoma	100% (3/3)
CLL	100% (24/24)
Lymphoplasmacytic lymphoma	100% (6/6)
Marginal zone B-cell lymphoma	100% (48/48)
Plasma cell myeloma	100% (1/1)
Hairy cell leukaemia	50% (1/2)
Follicular lymphoma	55% (6/11)
Diffuse large B-cell lymphoma	82% (9/11)
Hodgkin lymphoma	50% (1/2)

existence of IgH gene rearrangements or the status of the IgH gene in the special lymphoma types, especially the occurrence of somatic hypermutation in variable-region genes.

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