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The significance of MUM1/IRF4 protein expression and IRF4 translocation of CD30(+) cutaneous T-cell lymphoproliferative disorders: A study of 53 cases

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ABSTRACT

Current laboratory technics, clinicopathologic findings cannot always reliably distinguish primary cutaneous CD30(+) lymphoproliferative disorders (LPD), such as lymphomatoid papulosis (LyP), primary cutaneous CD30(+) anaplastic large cell lymphoma (PCALCL), transformed mycosis fungoides (T-MF) and systemic ALK(−) anaplastic large cell lymphoma (ALCL) with skin involvement. We investigated the presence of IRF4 translocation with break apart DNA-FISH method of these entities according to the recent studies of Feldman et al.

In our study group with 53 cases, the detection of IRF4 translocation had a specificity and positive predictive value for PCALCL of 100%. In contrast MUM1/IRF4 protein expression was distributed widely without any predictive value.

1. Introduction

Current laboratory technics, clinical and morphologic findings cannot always reliably distinguish primary cutaneous CD30(+) lymphoproliferative disorders (LPD), such as lymphomatoid papulosis (LyP), primary cutaneous CD30(+) anaplastic large cell lymphoma (PCALCL), transformed mycosis fungoides (T-MF) and systemic ALK(−) anaplastic large cell lymphoma (ALCL) with skin involvement. Differentiation of LyP and PCALCL can pose diagnostic challenges in certain cases. Besides histologic and immunophenotypic features, accurate clinical history is essential. However, the history is not always available in a routine setting. As a result, sometimes long periods of follow up are needed for an exact diagnosis. Moreover, the morphology in the lesions of PCALCL and T-MF may be identical. Distinguishing PCALCL from its systemic counterpart rests on clinical staging. However, even after the clinical staging, it may still be unclear. An isolated regional lymph node involvement may be a finding without any prognostic significance in PCALCL. On the other hand, it may point out a systemic lymhoma, as well. Feldman et al. identified the presence of translocation involving the multiple myeloma oncogene-1 (MUM1)/interferone regulatory factor-4 (IRF4) locus on 6p25 in peripheral T-cell lymphomas [1]. IRF4 is a transcription factor expressed in activated T cells, as well as plasma cells, some B cells, and their corresponding malignant counterparts [2]. In recent studies, the presence of IRF4 translocation with break apart DNA FISH method was discovered to be a valuable tool in distinguishing these cutaneous T cell lymphomas, expressing CD30 antigen [3–5]. The aim of our study was to investigate the value of IRF4 translocation and MUM1/IRF4 expression in skin biopsies.

2. Materials and methods

2.1. Case selection

Our study group was composed of 54 skin biopsies from 53 patients with cutaneous lymphomas, diagnosed according to the 2008 World Health Organization (WHO) classification [6]. The cases analyzed for this study were 26 LyP, 13 PCALCL, 9 T-MF, displaying CD30(+) large cells. In total, there were 11 cases of T-MF in our achieves, containing more than 25% of large cells, however only 9 of them demonstrated CD30 immunreactivity. CD30(−) T-MF cases were excluded. There were 2 biopsies from one case of PCALCL. Three cases of systemic ALK(−) ALCL, 1 systemic ALK(+) ALCL and 1 classic Hodgkin Lymphoma (CHL) were included as a control group. H&E and immune stained slides of all cases were histologically and clinically reviewed. The follow-up data were obtained from a combination of chart reviews and telephone interviews. Clinical information included age, gender, the site(s) of disease, the number and type of lesions, duration of follow-up, status at last follow-up, extracutaneous spread and death, if any. None of the patients of PCALCL had any systemic disease at the time of diagnosis during routine evaluation, which included complete blood cell count, physical and radiologic examination, as well as bone marrow biopsy performed in some cases.

We developed a dual color break apart FISH probe from related BAC clones for the detection of IRF4 rearrangements from tissue samples. We performed IRF4 FISH with tissue break apart dual color probe and at the same time immunohistochemistry for the expression of MUM1/IRF4.

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2.2. Florescence in situ hybridization

We used the National Center for Biotechnology Information (NCBI)'s database for the determination and selection of IRF4 related BAC clones. We checked the cytogenetics location of each BAC clone related to the IRF4 gene from the NCBI Map viewer. CTD-2308G5 (5′ IRF4-flanking BAC clone) and RP11-164H16 (3′ IRF4-flanking BAC clone) BAC clones were selected and ordered from Invitrogen (Life Technologies, USA). The break point is lying between the 3′ end of CTD-2308G5 and the 5′ end of RP11-164H16 BAC probes, suggesting the existence of a major breakpoint region and spanning almost 130 kb [4].

BAC clones were plated and propagated and glycerol stocks were prepared. A standard alkaline lysis procedure was used to isolate BAC DNA. The DNA was purified using the High Pure PCR Clean-up Micro Kit (Roche Applied Science, USA). FISH probes were created using purified CTD-2308G5 and RP11-164H16 BAC DNA's and labeled with Spectrum Green dUTP (Abbott Molecular Inc., USA) and Cyanine-3 dUTP (Enzo Life Sciences, Inc., USA) respectively, using Nick Translation Reagent Kit (Abbott Molecular Inc., USA). The labeled DNA probes were tested on interphase and metaphase cells and tissue samples for the control of chromosomal location and signal quality.

Tissue sections were placed on positively charged slides with 4–6 μm and deparafinized using the Depamiks Tissue Fish Deparafinization and Pretreatment Kit (Medimiks Biotechnology, Turkey). They were digested in enzyme working solution for 15 min at 37 °C. After enzymatic pretreatment, slides were immersed in 2× SSC solution (Abbott Molecular, USA) for 2 × 3 min, followed by dehydration in graded ethanol for 3 min (ethanol 70%, 85%, 99.9%) respectively and dried at RT.

The hybridization mix was prepared (2 μl dual color break apart IRF4 FISH probe and 8 μl miksish™ FISH Hybridization Buffer (Medimiks Biotechnology, Turkey) for each slide). Hybridization was performed in a humidified and airtight chamber at 37 °C for 16 h.

After hybridization, the coverslips were removed and slides washed in Wash Solution I (containing 2% 20× SSC, NP-40 (conc. 0.3%)) in a 73 °C water bath for 2 min and then in Wash Solution II (10% 20× SSC, NP-40 (conc. 0.1%)) for 10 s.

Totally dried slides were then counterstained with 10 μl DAPImiks™ (4′,6-diamidino-2-phenylindole dihydrochloride) (Medimiks Biotechnology, Turkey). The slides were kept at −20 °C for at least 15 min to optimize the antifading effect before microscopy and analyzed using the Duet, Automated Combined Mass Scanning and Analysis System (Bioview, Israel).

Cells with two fusion signals were considered normal cells without translocation. Cells with one fusion, one green, and one red signal (split signal pattern) were considered positive for translocation, when the distance between green and red signals was more than or equal to 1.5 μm. When the distance between the split signals was between 1 and 1.5 μm, it was classified as suspect for positive and when the distance was less than 1 μm or equal to 1 μm, it was classified as normal.

2.3. Immunohistochemistry

Formalin-fixed paraffin-embedded tissue sections were a incubated in 1 mM EDTA buffer (pH 8.0) for 60 min for pretreatment and then reacted with MUM1/IRF4 (MUM1 S clone EAU32, Novacastra) at a dilution of 1:100 for 80 min at 37 °C. Ventana Benchmark XT immunostainer was used and signals were detected using the Ultraview Universal DAB Kit. MUM1/IRF4 staining was evaluated together with the CD30 antibody stained sections. A positive MUM1/IRF4 staining was defined as a nuclear labeling in more than 10% of the large cells and semiquantitatively scored as follows: −, zero or less than 10% of large tumor cells; +, 10–50% of the stained large tumor cells; ++, 50–85% of the stained large tumor cells; +++, >85% of the stained large tumor cells [4].

2.4. Statistics

Fischer's exact test was used to evaluate the differences observed in the frequency of IRF4 translocation and MUM1/IRF4 expression.

3. Results

The clinical information of all the patients, except the control group, is given in Table 1.

All cases of LyP were characterized by skin lesions that typically wax and wane, leaving atrophic scars. The most common type of lesion was papules (54%). The distribution of the lesions was mostly generalized (64%). The median age was 41 years (range, 1–78 years), with male:female (M:F) ratio 1:1. Out of 26 cases of LyP, 21 were type A (81%), while 5 were type C (19%). Within a follow-up period of 1–96 months (median 25 months), 8 (31%) cases still had LyP lesions, while 5 (19%) cases were without lesions at the last follow-up. None of the cases had extracutaneous involvement.

Table 1
The clinical data of all patients.

	LyP (n = 26)	PCALCL (n = 13)	T-MF (n = 9)
Gender			
Female	13 (50%)	7 (54%)	5 (55%)
Male	13 (50%)	6 (46%)	4 (45%)
Age			
Range	1–78	27–85	21–56
Median	41	62	38
Type of lesions			
Papule	14 (54%)	3 (24%)	–
Papulonodule	4 (15%)	1 (7%)	–
Nodule	8 (31%)	8 (62%)	–
Tumor	–	–	5 (56%)
Plaque	–	–	4 (44%)
Not known	–	1 (7%)	–
Number of lesions			
5>	5 (19%)	6 (46%)	–
5–10	3 (12%)	2 (15%)	1 (11%)
10<	17 (65%)	5 (39%)	8 (89%)
Not known	1 (4%)	–	–
Site of lesions			
Generalized	15 (58%)	1 (7%)	7 (78%)
Localized	11 (42%)	8 (62%)	2 (22%)
Solitary	–	4 (31%)	–
Extracutaneous spread			
Present	–	1 (7%)	3 (33%)
Not present	25 (97%)	11 (86%)	6 (67%)
Not known	1 (3%)	1 (7%)	–
Therapy			
Total exicion	–	1 (7%)[a]	–
PUVA/UV-B	4 (15%)	–	–
Topical corticosteroid	3 (12%)	1 (8%)	–
Chemotherapy (CT)	–	4 (31%)	3 (33%)
Radiotherapy (RT)	–	4 (31%)	–
CT + RT	–	2 (15%)	2 (22%)
Without therapy	2 (8%)	–	–
Not known	17 (65%)	2 (15%)	4 (45%)
Duration of follow up (month)			
Range	1–96	12–288	2–60
Median	25	97	20
Status at last follow up			
Stable disease	8 (31%)	4 (31%)	4 (45%)
Complete remission	5 (19%)	5 (38%)	–
Died of disease	–	–	2 (22%)
Died of other reasons	–	–	–
Not known	13 (50%)	4 (31%)	3 (33%)

[a] After total exicion, RT was performed in one case.

In cases of PCALCL, the patients primarily experienced nodules (62%), either localized (62%) or solitary (31%). The median age was 62 years (range, 27–85 years), with M:F ratio 1:1. In a follow-up period between 12 and 288 months (median 97 months), only one patient had regional lymph node involvement without any further dissemination. In the last follow-up, 4 cases (31%) were living with lesions, while 5 cases (38%) were without.

Out of 9 MF cases with CD30(+) large cell transformation, 3 cases (33%) were in the plaque stage and 5 cases (56%) were in the tumor stage. The remaining case of T-MF, was a folliculotropic MF (11%). The median age was 38 years (range, 21–56 years), with M:F ratio 1:1. CD30(+) large cells varied from 20 to 50% among the dermal infiltration in cases of T-MF.

3.1. MUM1/IRF4 expression

Among 26 cases of LyP, the expression of MUM1/IRF4 was present in 16 cases (61.5%). Out of 21 cases of LyP type A, 9 cases