Fluorescence in situ hybridization analysis of the *hTERC* region in acute myeloid leukemia patients

Akut myeloid lösemi olgularında floresan in situ hibridizasyon yöntemi ile hTERC bölgesinin analizi

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Abstract

Objective: The telomerase RNA component (*hTERC*) gene is located at 3q26. Increased *hTERC* gene expression has been frequently observed and amplification was shown using fluorescence in situ hybridization (FISH) in different cancers. The aim of this study was to determine whether *hTERC* gene amplification is detectable by FISH in acute myeloid leukemia (AML) cells.
Material and Methods: FISH and karyotype results at the time of diagnosis of 23 adult AML patients were retrospectively evaluated. Additionally, fixed cells were hybridized with an hTERC region-specific FISH probe to determine gene amplification.
Results: Ten of the 23 patients had a normal karyotype and 6 had an abnormal karyotype. *hTERC* region amplification was not observed in any of the patients.
Conclusion: Although it was reported that *hTERC* gene amplification may partially contribute to increased telomerase expression and activity in leukemic cells, it is not possible to make such a conclusion based on the results of the this study, as *hTERC* amplification was not observed in the study group. This suggests that increased telomerase activity via gene amplification in the development of AML may not be as important a factor as it is in solid tumors. *(Turk J Hematol 2011; 28: 103-6)*
Key words: AML, *hTERC*, FISH

Received: February 11, 2010 *Accepted: September 6, 2010*

Özet

Amaç: Telomeraz RNA komponenti (*hTERC*) geni 3q26 bölgesinde yer alır. Çeşitli kanserlerde artmış *hTERC* gen anlatımı sıklıkla saptanmış ve FISH yöntemi ile gen amplifikasyonu gösterilmiştir. Bu çalışmanın amacı akut miyeloid lösemi (AML) hücrelerinde *hTERC* gen amplifikasyonunun FISH yöntemi ile saptanabilirliğinin araştırılmasıdır.
Yöntem ve Gereçler: Bu çalışmada 23 yeni tanı erişkin AML olgusunun FISH ve karyotip sonuçları geriye dönük olarak değerlendirildi. Ayrıca gen amplifikasyonunu değerlendirebilmek için tespit edilmiş hücreler *hTERC* bölgesine özgül FISH probu ile melezlendi.

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doi:10.5152/tjh.2011.25

Bulgular: Çalışma kapsamına alınan 23 hastanın 10'unda normal karyotip; 6 hastada anormal karyotip bulundu. Hastaların hiçbirinde *hTERC* bölgesinde gen amplifikasyonu saptanamadı.
Tartışma: Hasta grubumuzda *hTERC* amplifikasyonu bulmadık. Bu nedenle, *hTERC* gen amplifikasyonunun lösemik hücrelerde telomeraz anlatımı ve aktivitesini arttırmada kısmen etkili olduğunun bildirilmesine karşın, çalışmamızın sonuçları ile benzer bir sonuca ulaşmak mümkün olmamıştır. Bu sonuç, AML gelişiminde gen amplifikasyonu yoluyla telomeraz aktivite artışının, solid tümörlerde olduğu kadar önemli bir faktör olmadığı görüşünü destekler niteliktedir. *(Turk J Hematol 2011; 28: 103-6)*
Anahtar kelimeler: AML, *hTERC*, FISH

Geliş tarihi: 11 Şubat 2010 *Kabul tarihi: 6 Eylül 2010*

Introduction

Acute myeloid leukemia (AML) originates from myeloid cells. The disease is characterized by rapid proliferation of abnormal cells, which accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common form of acute leukemia in adults and its incidence increases with age [1]. Morphological, cytochemical, immunophenotypic, cytogenetic, and molecular features of the blasts are important factors in the classification of AML patients [2].

Telomeres, located at the ends of chromosomes, determine the replication capacity of cells. They shorten during each cell division and it is generally thought that telomere length serves as a clock for cells. Telomerase is a ribonucleoprotein complex, consisting of human transcriptase reverse transcriptase (hTERT), proteins (hTP1), and RNA template for telomeric DNA synthesis (hTERC) [3]; this complex synthesizes telomeres and stabilizes their length. Mutations in any of these components may result in a rare bone marrow failure syndrome known as dyskeratosis congenita [4].

Telomerase activity was reported to be low in normal human somatic cells [3]. Proliferating cells become senescent when telomeres are short and when there is no telomerase activity. Cancer cells are known to overcome this senescence pathway and become immortalized. Immortalization is almost always accompanied by expression of telomerase, which is most likely necessary for the continued growth of cancer cells. Telomerase is activated in most malignant tumours, but is usually inactive in normal somatic cells [3]. Despite the questionable role of telomerase reactivation in cell immortalization and carcinogenesis, telomerase may serve as a diagnostic marker for tumor development [5]. Several studies have shown that telomerase activity can be used to differentiate malignant from normal tissue in various organs; however, the majority of reports are devoted either to telomerase activity or expression [6]. The present study aimed to determine the presence of amplification of the hTERC component of the telomerase complex in cultured bone marrow cells obtained from AML patients.

Materials and Methods

The study included bone marrow samples taken at the time of diagnosis from 23 AML adult patients between 2006 and 2008 that analyzed in our cytogenetics laboratory. Conventional cytogenetic analysis and FISH results for each patient were evaluated retrospectively, and additional hybridization with the hTERC (3q26)/ 3q11 probe (Kreatech, The Netherlands) was performed to determine hTERC gene amplification. Slides were dehydrated in 70%, 85%, and 90% alcohol series, consecutively, and dried at room temperature. Denaturation and hybridization were carried out in a HyBrite denaturation/ hybridization system for FISH (Vysis, UK). The probe was denatured at 75°C for 5 min. Hybridization was carried out at 37°C for 14-16 h. After hybridization, the slides were washed in first post hybridization wash buffer at 73°C for 2 min, and then in second post hybridization wash buffer at room temperature for 1 min and counterstained with DAPI.

Signals were quantified in ≥200 cells for both *hTERC* gene and 3q11 signals under oil immersion at 1000× magnification using the recommended filters. Results are expressed as the ratio of *hTERC* signal (orange) to 3q11 signal (green). The expected ratio is 1:1; which indicates there is no gene amplification and when the ratio of signals is ≥2:1, it indicates *hTERC* gene amplification (Figure 1). Our samples were chosen from archive material consisting of patient samples who approved material useage after diagnostic procedures.

Results

Among the 23 patients, 14 (60.9%) were male and 9 (39.1%) female; median age was 49 years (range: 15-76 years). Conventional cytogenetic and routine FISH results are given in the Table 1. Cytogenetic analysis was not possible in 7 patients due to culture failure or because we did not find suitable metaphases for chromosome analysis (shown in the Table as N/A). In all, 10 of the 23 patients had a normal karyotype and 6 had an abnormal karyotype. The observed abnormalities included trisomy 8, t(1;3), der(7), t(15;17), del(5), and inv(16). We did not observed any complex karyotypes and the above-mentioned cytogenetic defects were the only abnormality in each patient. Routine FISH analysis showed that 5 patients had t(15;17), 2 patients had t(8;21), 1 patient had triple signal for the 8q22 region, 1 patient had a deletion in the 5q31 region, and 1 patient had inv(16). According to FISH analysis, none of the patients had *hTERC* gene amplification.

Discussion

Normal human cells have low levels of telomerase expression; however, when telomere length reaches a critical point abnormal activation of telomerase can lead to immortalization and uncontrolled proliferation. This process is associated with the development of many leukemias and lymphomas [8]. More than 80% of various cancers have increased telomerase activity, and it has been reported that amplification of the telomerase gene might play a role in telomerase reactivation, which leads to cell immortalization and seems to be a common event in carcinogenesis [9].

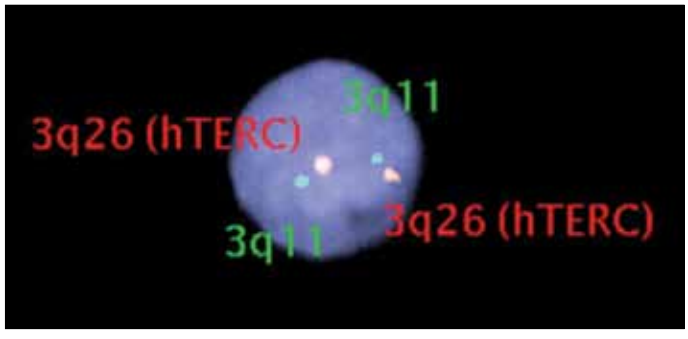

Figure 1. Normal AML cells for *hTERC* gene amplification analysis using FISH. The specific molecular probe for *hTERC* was labeled with rhodamine (Spectrum Orange) and the 3q11 reference probe was labeled with fluorescein (Spectrum Green)

It has been reported that increased *hTERT* and/or *hTERC* gene expression may be an important mechanism involved in upregulated telomerase activity. Accordingly, investigation of *hTERT* and/or *hTERC* amplification might be useful in the diagnosis and follow-up of cancer patients [10]. In the present study we only examined amplification of the *hTERC* component, as it is known that *hTERC* gene amplification alone can result in increased telomerase activity [10]. Further investigation of *hTERT* amplification may be useful for elucidating the interactions of these genes in the progression of AML.

hTERC gene expression can be affected by and regulated by various processes inside cells; namely, gene amplification, transcriptional regulation, and epigenetic modification [11]. It was suggested that the high-level of expression and activity of telomerase observed in acute leukemia cells might be due to *hTERC* gene amplification, which is localized at 3q26.3. FISH is a useful method for observing gene amplification and was used in the present study to determine *hTERC* gene amplification. Previous studies have reported that amplification of *hTERT* and *hTERC* genes in acute leukemia cells exhibit a variable number of copies-as many as 12-based on FISH analysis [12].

One study that included patients with different types of leukemia subgroups reported that amplification of *hTERC* and *hTERT* genes was associated with increased telomerase activity and expression in leukemia cells [12]. Hematologic malignancies differ from other cancers, as they do not originate from cells with low-level telomerase activity. Serakinci et al. reported that there was no amplification of the *hTERT* region in patients with monosomy 7 and no deletion at 7q31 [13]. In the present study 1 patient had a derivative chromosome 7 (patient No. 10), which we think originated from a translocation with an undefined chromosome. The fact that we did not observe amplification of the *hTERC* region in this patient supports the notion that this amplification may not be a characteristic of leukemia cells with an abnormality in chromosome 7. Nonetheless, the unknown chromosomal component of the translocation and the putative effector genes on the derivative chromosome 7 should not be overlooked.

Table 1. The patients, and their conventional cytogenetic and FISH results

Patient Number	Sex	Age	Karyotype	FISH panel
1	E	76	N/A	Normal
2	E	15	47,XY,+8	8q22 triple signal
3	E	24	46,XY,t(1;3) (p36.3;q21)	Normal
4	K	64	N/A	Normal
5	E	27	Normal	Normal
6	K	28	N/A	t(15;17) +
7	K	34	N/A	t(15;17) +
8	K	50	Normal	t(15;17) +
9	E	49	Normal	Normal
10	E	47	46,XY,der(7)t(7;?) (q32;?),t(8;21)(q22;q22)	t(8;21) +
11	E	33	46,XY,t(15;17)(q22;q21)	t(15;17) +
12	E	55	N/A	Normal
13	E	52	N/A	t(8;21) +
14	E	37	46,XY,del(5)(q31) [16]/46,XY[2]	Deletion 5q31
15	K	50	Normal	Normal
16	E	75	46XY,inv(16) (p13q22) [4]/46,XY[1]	inv(16) +
17	E	27	Normal	Normal
18	E	46	N/A	Normal
19	E	53	Normal	Normal
20	K	39	Normal	Normal
21	K	57	Normal	Normal
22	K	58	Normal	t(15;17) +
23	K	65	Normal	Normal

Given the genetic heterogeneity of AML, investigation of *hTERC* gene amplification and frequent genetic changes could further delineate the tumor biology and might be useful for determining the prognostic factors in AML. In the present study *hTERC* gene amplification in the bone marrow samples was not observed, based on FISH analysis. Given the limited number of cases included, it is not possible to definitively conclude that *hTERC* gene amplification has any impact on the clinical course of AML or that its amplification based on FISH could be used as a diagnostic marker; however, the present results are in agreement with those of previous studies that have suggested that increased telomerase activity due to telomerase gene amplification may not be as influential in leukemias as in solid tumors [13]. Additional research (with larger patients groups) on the expression of the *hTERC* gene and other components of the telomerase complex is warranted.

Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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