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Telomerase Activity And Sialic Acid Levels In Human Glioma Cell Lines

Insan Glioma Hücre Dizilerinde Telomeraz Aktivitesi ve Siyalik Asit

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ABSTRACT: ***Objective:*** The telomerase enzyme complex comprises among other proteins, two main subunits; TERT and TR component. Polysialic acid chains modify the adhesive function of NCAM molecules, presumably by making it easier to relinquish the cells to which they are stuck. Telomerase activity and sialic acid levels may be used to predict the biological behavior of gliomas**.**
Materials and Methods: Telomerase activity and sialic acid levels were investigated in the cell extracts taken from human glioma cell lines. To detect telomerase activity, telomeric repeat amplification protocol was performed. To examine sialic acid levels, a Sialic Acid Quantification Kit was used. The expression of hTERT, hTR and GAPDH in cell line, respectively, was analyzed by using a RT-PCR kit. Cells fixed on the flask base were used for NCAM through the streptavidin-biotin-peroxidase staining method.
Results: Telomerase activity was found to be increased in the later passages of human glioma cells, although it demonstrated a fluctuating pattern. Telomerase RNA and TERT expression were positive in all passages. Sialic acid levels were also seen to be increased, showing regular rising in the consecutive passages of human glioma cell line. Immuno-staining of the NCAM molecule was positive.
Conclusion: The telomerase activity, which has been initially expected to increase in the proceeding passages, was increased by manifesting undulation. Besides, the fact that sialic acid levels showed a regular increase gradually in the proceeding passages of the glioma cell line may indicate that this increase is more regular than that of the telomerase activity.
Key Words: DK-MG cell line, sialic acid, telomerase activity, hTERT, hTR, NCAM

ÖZET: ***Amaç:*** Telomeraz enzim kompleksi, diğer proteinlerin yanında, TERT ve TR olmak üzere iki temel subunitten oluşur. Polisiyalik asit zincirleri muhtemelen hücrelerin birbirlerine daha kolay yapışmasını sağlayarak, NCAM moleküllünün adeziv fonksiyonunu düzenler. Telomeraz aktivitesi ve siyalik asit düzeyleri gliomaların biyolojik davranışlarını belirlemede kullanılabilir.
Gereç ve Yöntem: İnsan glioma hücre dizilerinden elde edilen hücre ekstraktlarında, telomeraz aktivitesi ve siyalik asit düzeyleri araştırıldı. Telomeraz aktivitesini belirlemek için, telomerik tekrar amplifikasyon protokolü uygulandı. Siyalik asit düzeylerini ölçmek için, siyalik asit kiti kullanıldı. Hücre dizisinde hTERT, hTR ve GADPH ekspresyonları RT-PCR kiti kullanılarak belirlendi. NCAM için, flask tabanında fikse edilen hücrelerde streptavidin-biotin peroksidaz boyama metodu uygulandı.
Bulgular: İnsan glioma hücre dizilerinin sonraki pasajlarında telomeraz aktivitesi her ne kadar dalgalı olsada artmış olarak bulundu. Telomeraz RNA'sı ve TERT ekspresyonları tüm pasajlarda pozitifti. Siyalik asit düzeyleri de artış gösterdi ve bu artış insan glioma hücre dizilerinin ilerleyen pasajlarında düzenliydi. NCAM molekülü tüm pasajlarda pozitif olarak bulundu.
Sonuç: Başlangıçta ilerleyen pasajlarda artması beklenen telomeraz aktivitesi dalgalı olarak artmıştır. Bunun yanında glioma hücre dizilerinin ilerleyen pasajlarında siyalik asit düzeyleri daha düzenli artış göstermektedir.
Anahtar Kelimeler: DK-MG hücre dizisi, siyalik asit, telomeraz aktivitesi, hTERT, hTR, NCAM

INTRODUCTION

Human chromosomes have telomeric repeat sequences of TTAGGG at the end. Telomerase is a large ribonucleoprotein complex and elongates telomeric DNA by these repeats. Cancer cell DNA is continuously extended or maintained by telomerase to compensate for the loss of telomeric repeats, and the cells thus become immortalized (1-5).

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Telomerase activity can be used as a tumor marker, and the activation of telomerase may be also correlated with the malignant progression of gliomas (1,6-10). The telomerase complex comprises among other proteins, two main subunits; telomerase reverse transcriptase (TERT) and a functional RNA component (TR) (11-14). TERT and TR may also be considered as distinctive factors in understanding malignant progression in gliomas (15,16).

Sialic acid (N-acetylneuraminic acid) is a considerable component of gangliosides, which fundamentally consolidate cellular membranous structure and play a role in the maintenance of intercellular activity (17,18). It has been proposed that cellular sialic acid levels could be used as a tumor marker in malignancies and cancer (19,20). Sialic acid expressions have been also detected in gliomas (20-23). Polysialic acid chains (PSA) modify the adhesive function of neural cell adhesion molecules (NCAM), presumably by making it easier to relinquish the cells to which they are stuck. NCAM is also a dominant carrier of PSA and has an important role in regeneration (18,23-25).

Gliomas vary in growth potential, extent of invasiveness, tendency for progression and clinical course. Investigations have been focused on understanding the cellular and molecular basis of their malignant progression.

Both telomerase activity and sialic acid levels can be used to understand the malignant potential of gliomas. The purpose of the present paper is to compare telomerase activity and cellular sialylation in human glioma cell line (DK-MG). To achieve this, telomerase activity, expression of TR and TERT, sialic acid levels, and the existence of NCAM in cell membrane were evaluated in consecutive cell extracts from human glioma cell line.

MATERIALS AND METHODS

This study was supported by grant of the Research Foundation of Eskişehir Osmangazi University, Turkey.

The human glioma cell line [(DK-MG), Deutsche Sammlung Von Mikroorganismen und zellkulturen (DSMZ), Braunschweig, Germany] was grown in Dulbecco's Eagle's minimal essential medium [(DMEM) (Sigma-Aldrich)] supplemented with 20% fetal bovine serum [(FBS) (Biological Industries)] and penicillin-streptomycin (Sigma-Aldrich). Cells from passage 3rd, 6th, 9th, 12th and 15th were trypsinised, counted, and their viability was determined by trypan blue exclusion assay. Following this, telomerase activity and sialic acid levels were measured three times at each passage, respectively. Results were achieved through these measurements. In the same passages, TERT and TR expression were also examined and immunohystochemical staining for NCAM was carried out.

To detect telomerase activity, cell lysate was extracted by lysis buffer, and telomeric repeat amplification protocol was performed (26), using a PCR-based TRAP cells were collected, and after the PCR and hybridization-ELISA process, hybridization-ELISA in a microtiter plate and reader (A450nm-A690nm) was used to read the telomerase activity. To examine TeloTAGGG Telomerase PCR ELISA^{PLUS} kit (Roche). Those samples containing $2x10^5$ sialic acid levels, a Sialic Acid Quantification Kit (Sigma-Aldrich) was used in the same passages that had been used for measurements of telomerase activity. Following spectrophotometric analysis (A340 nm), sialic acid levels were detected in the cell extracts containing $2x10^6$ cells.

Total RNA was isolated from cultured cells ($6x10^7$ cells) using an Ez-RNA Total RNA Isolation kit (Biological Industries). The expression of hTERT, hTR and control glyceraldehydes 3-phosphate dehydrogenase (GAPDH) mRNA in DK-MG cell line, respectively, was analyzed by RT-PCR (Reverse transcriptase-PCR) using a RT-PCR kit (Qbiogene) (11,27). The reaction products were subjected to electrophoresis in agarose gels and visualized by ethidium bromide staining (27).

Cells fixed on the flask base were used for immunohystochemical analysis through the streptavidin-biotin-peroxidase staining method. A Hystostain SP kit (Zymed) and its antibody (NCAM, CD56 Diagnostic Biosystems) were used for this analysis. In all cell passages, enhancement of staining was evaluated for the expression of NCAM. Assessment of the results was analyzed in a double-blind study. Evaluations of the stained preparations were made using light microscopy by three of the authors and scored independently.

Statistical Analysis

All data were expressed as means ± standard deviation. Differences between the means of various results were assessed for statistical significance by ANOVA, followed by Tukey's multiple comparison tests. A p value < 0,05 was considered to indicate statistical significance.

RESULTS

In the DK-MG cell lines, telomerase activity was seen to be increased in the 6th (p<0.05), 12th (p<0.01) and 15th passages (p<0.001) when compared with the 3rd passage, and there was no statistical increase in the 9th passage compared with other passages (Table 1).

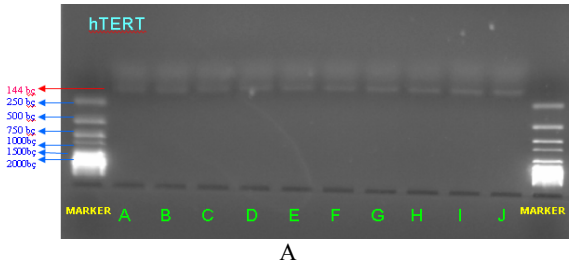
Table 1: *Telomerase activity and sialic acid levels of each passage. Values are expressed as means ± standard deviation. n: number of measurements at each passage.*

Passages	n	Telomerase Activity	Sialic Acid Level (nmol)
3rd P	3	**0,12 ± 0,01**	**1,93 ± 0,27**
6th P	3	**10,94 ± 2,24***	**9,11 ± 3,90**
9th P	3	**0,19 ± 0,05**	**9,00 ± 2,41**
12th P	3	**14,52 ± 3,71****	**19,78 ± 0,16**
15th P	3	**25,11 ± 6,93*****	**45,62 ± 20,39****

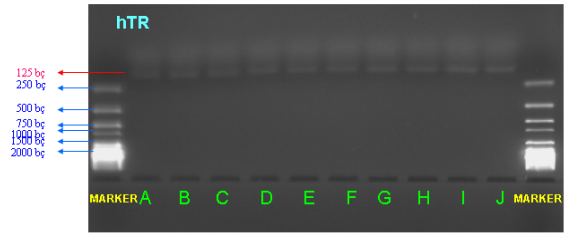
*p<0.05, **p<0.01, ***p<0.001

Sialic acid level in the DK-MG cell lines was statistically increased in the 6th, 9th, 12th and 15th passages when compared with the 3rd passage. An increase in sialic acid level in the 15th passage was considered to be statistically significant compared with the 3rd, 6th, 9th (p< 0.01) and 12th passages (p<0.05) (Table 1).

Cells showed consistent TR and TERT expression patterns in successive passages of DK-MG cell line (Figure 1 A,B). Immunohystochemical staining of NCAM was also positive in all passages (Figure 2 A,B).

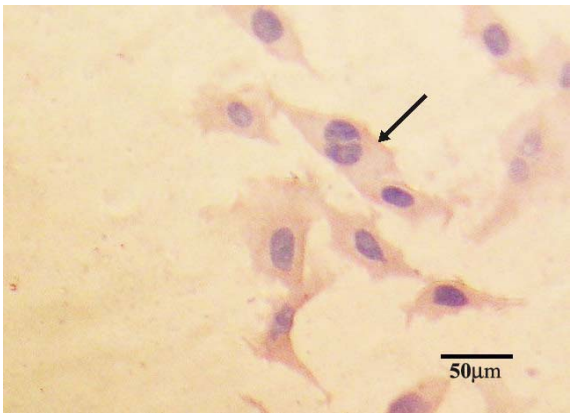


A

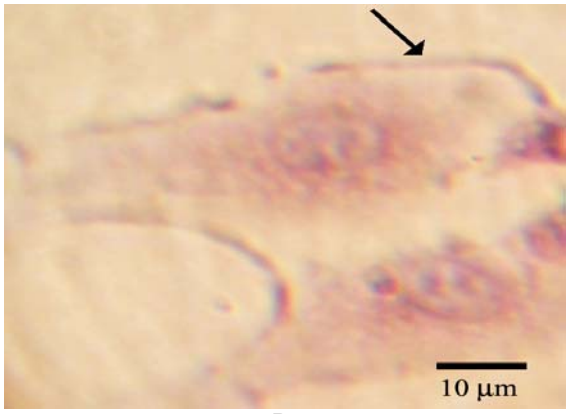


B

Figure 1: TERT (A) and TR (B) expressions in DK-MG cell line (A-J: samples, bp: base pairs).



A



B

Figure 2 A, B: Photomicrographs showing immunohystochemical staining of NCAM (arrows) in DK-MG cell membranes.

DISCUSSION

The telomerase activity, generally positive in the cancer cells, increases in cell line with malignancy and aggressiveness (2,5,28,29). Telomerase activity has been manifested as positive in 11 (85%) of the 13 malign pancreas tumors, and as negative in 15 benign pancreas tumors. In accordance with these results, it has been claimed that the telomerase activity in pancreas tumors is an important parameter in determining the malignancy (30). Of the 62 patients with breast cancer of which the malignancy has not been determined, 50 of the specimens taken via fine needle aspiration method have showed an increase in the telomerase activity. Authors have asserted that the telomerase activity may be used with 81% accuracy in determining the breast cancer malignancy (31). Telomerase activity has been established as positive in 10 of the 12 cervical cancers, 12 of the 13 endometrial cancers,

18 of the 21 ovarian cancers, 2 of the 2 tubal cancers, and 1 of the 1 vulvar cancer case. Based on this result, it has been claimed that the telomerase activity could be used in determining the malignancy of 88% of the gynecologic malign tumors (32). In another study that focused on the telomerase activity in ovary tumors, the telomerase activity has been found in 23 (92%) of the 25 malignant ovary tumors, 1 (16.7%) of the 6 borderline malign tumors, and 2 (20%) of the 10 benign tumors are higher compared to other tumors (33). In 41 specimens with soft tissue tumors, it has been indicated that the telomerase positive cells were not only malignant but also aggressive (34). Furthermore, they have showed a relationship between the metastasis and the increasing of telomerase activity in locally recurring tumors. Of the malign gliomas, telomerase activity of 45% and 89% was determined in anaplastic astrcytoma (grade III) and in glioblastome multiforme (grade IV) respectively, and it has been also reported that the increase in the telomerase activity showed the malignancy of these tumors (12).

Although many studies have been carried out on the telomerase activity in tissues, this issue has been studied less on the cell lines. In a study on the cell lines, the telomerase activity and hTERT expression have been examined in LN-18, U138MG, U87MG, LN-428, D247MG, T98G, LN-319, LN-229, A172, U251MG, U373MG, LN-308 glioma cell lines and in normal astrocyte TEN cell line. Telomerase activity and hTERT expression have been established as positive in all of the 12 glioma cell lines, and as negative in the normal cell line (35). Consecutive cell line passages in immortal embryonic esophageal epithelium cell line (SHEE) have been investigated without administering any average substances, and researchers have showed that the cells were differentiated and become cancerous in the 31st passage, that they became premalignant cells in the 61st passage, and that they manifested strong potential and fully malignant transformation in the 85th passage. This study has showed that the transformation in the passage to passage cells takes the cells to malignancy (36). Moving from the fact that the telomerase activity would increase along with malignancy, the result of our study from the view of telomerase activity proves to be in accordance with these studies.

In addition to telomerase activity, TERT and TR expressions responsible for this activity are also positive, and their increasing is observed in the malignant progression in cancerous cell lines (37,38). While hTERT expression has not been seen in the normal fibroblast cell lines (MRC5), its expression has been determined as positive in 6 different malignant glioma cell lines (U87-MG, A172, T98G, U373-MG, U251MG, GB-1)(12). In our results, TERT and TR expressions were seen as positive also in the passages where telomerase activity decreased. This supports the fact that telomerase activity exists in the medium, but that even decreases are possible in glioma line which are definitely cancerous.

In a report manifesting that sialic acid levels increase during cancer, serums of patients with 50 gastrointestinal system cancers and 20 controls have been investigated, and it has been determined a significant sialic acid increase compared with the controls (39). Serum and bronchialveolar lavage sialic acid levels in 21 malignant and 10 benign lung cancer patients have been determined to be higher compared with the control group (40). The blood specimens of 37 primary tumor patients and 8 relapse larynx cancer patients have indicated an increase in the sialic acid levels, and this increase has been more significant in the relapse patients (41). In an investigation on 50 controls and 43 head-neck cancer patients, it has been determined that serum sialic acid levels in cancer patient increased compared with the control, and also this increase has been much higher in malignant progression (42). Much higher serum sialic acid levels of 35 patients with metastasis and 25 colorectal cancer patients have been shown compared with the group with no metastasis and the control (43).

There was no suggestion showing how the level of sialic acid changes from passage to passage in our cell lines. In the proceeding passages of the used cell line, sialic acid level increased gradually, and this increase was more regular compared with the telomerase activity. Due to the NCAM-PSA relationship, the existence of NCAM molecules could be shown in the present study. The telomerase activity, which has been initially expected to increase in the proceeding passages, was increased by manifesting undulation. Its use as a single parameter may be considered as a misleading factor for the researcher. Besides, the fact that sialic acid levels showed a regular increase gradually in the proceeding passages of the glioma cell line may indicate that this increase is more regular than that of the telomerase activity. The results of our study pointed out the necessity to check other parameters in addition to telomerase activity in future studies on cancer cell lines such as glioma. There is also need

to conduct many other studies on different cancer cell lines in order for our findings to become more definite.

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