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The Progression of Telomerase Activity And Sialic Acid Levels in Rat Glioma Cell Lines

Siçan Glioma Hücre Dizilerinde Telomeraz Aktivitesi ve Siyalik Asit

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ABSTRACT: ***Objective:*** Telomerase plays an important role in the cellular immortalization of cancers. It contains telomerase RNA, telomerase reverse transcriptase. Sialic acid consolidates the cellular membrane and plays a role in the maintenance of intercellular activity. Telomerase activity and sialic acid levels may be used to predict the biological behavior of C6 rat glioma cell lines.
Materials and Methods: Telomerase activity and sialic acid levels were investigated in consecutive cell line passages in cell extracts taken from C6 rat glioma cell line. Both telomerase activity and sialic acid levels were determined in the 3rd, 6th, 9th, 12th and 15th passages. To detect telomerase activity, cell lysate was extracted by lysis buffer, and telomeric repeat amplification protocol was performed, using a PCR-based TRAP TeloTAGGG Telomerase PCR ELISA PLUS plus kit. To examine sialic acid levels, a Sialic Acid Quantification Kit was used in the same passages that had been used for measurements of telomerase activity.
Results: Telomerase activity was found to be increased in the later passages of C6 cells, although it demonstrated a fluctuating pattern. Sialic acid levels were also seen to be increased, showing regular rising in the consecutive passages of C6 cell line.
Conclusion: In the consecutive passages of C6 cell line, an increase in sialic acid levels may tend to be regular, but an increase in telomerase activity may be irregular. During the follow-up of glioma cell line, sialic acid levels may represent a more reliable biologic prognostic factor.
Key Words: C6 cell line, sialic acid, telomerase activity

ÖZET: ***Amaç:*** Telomeraz kanser hücrelerinin ölümsüzlüğünde önemli bir rol oynar. Telomeraz, telomeraz RNA'sı ve telomeraz revers transkriptazdan oluşur. Siyalik asit, hücresel membranı güçlendirir ve hücreler arası aktivitede büyük rol oynar. Polisiyalik asit zincirleri, nöronal hücre adezyon molekülünün adeziv fonksiyonunu değiştirir. Telomeraz aktivitesi ve siyalik asit düzeyleri tümör belirleyicisi olarak kullanılabilir.
Gereç ve Yöntem: C6 sıçan glioma hücre dizisinden ard arda yapılan 3., 6., 9., 12. ve 15. pasajların ekstraktlarında, telomeraz aktivitesi ve siyalik asit düzeyleri incelendi. Telomeraz aktivitesini belirlemek için, TRAP TeloTAGGG Telomerase PCR ELISA plus, siyalik asit düzeylerini belirlemek için, Sialic acid quantification kiti, kullanıldı.
Bulgular: Telomeraz aktivitesi C6 hücre dizisinin pasajlarında, dalgalanmalar şeklinde bir artış gösterdi. Telomeraz RNA'sı ve telomeraz revers transkriptazın ekspresyonları tüm pasajlarda pozitif olarak izlendi. Siyalik asit düzeyleri, ilk pasajdan itibaren son pasaja doğru düzenli bir artış gösterdi.
Sonuç: C6 hücre dizilerinin ilerleyen pasajlarında siyalik asitin düzenli bir şekilde giderek artması, telomeraz aktivitesindeki artışın ise düzensiz olması, gliomaların klinik ve biyolojik davranışlarının belirlenmesinde, telomeraz aktivitesinden ziyade, siyalik asitin daha güvenilir biyolojik prognostik faktör olabileceğini düşündürmektedir.
Anahtar Kelimeler: C6 hücre dizisi, siyalik asit, telomeraz aktivitesi.

INTRODUCTION

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. The cell cycle is related to telomere length and telomerase activity. 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). Telomerase activity can be used as a tumor marker, and the activation of te-

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lomerase 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 (19,20). Sialic acid expressions have been also detected in gliomas (20-23). Both cellular 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 rat glioma cell lines. To achieve this, telomerase activity and sialic acid levels were evaluated in consecutive cell extracts from rat (C6) glioma cell lines.

MATERIALS AND METHODS

This study was supported by grant of the Research Foundation of Eskişehir Osmangazi University, Turkey. The rat glioma cell line [(C6) Deutsche Sammlung Von Mikroorganismen und zellkulturen (DSMZ), Braunschweig, Germany] was grown in RPMI medium (Sigma-Aldrich) supplemented with 10% FBS and penicillin-streptomycin. C6 cell line was maintained with 15 passages. Cells from passage 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} 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. To detect telomerase activity, cell lysate was extracted by lysis buffer, and telomeric repeat amplification protocol was performed (24), using a PCR-based TRAP TeloTAGGG Telomerase PCR ELISA plus kit (Roche). Those samples containing $2x10^5$ 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 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.

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

Telomerase activity: (Table 1, Figure 1) In the C6 cell lines, there was no statistical difference found in the 6^{th}, 9^{th} and 12^{th} passages when compared with the 3^{rd} passage, although telomerase activity in the 6^{th} passage did show an increase. In the 15^{th} passage of the C6 cell line, there was a statistical increase compared with the other passages ($p< 0.001$).

Sialic acid levels: (Table 1, Figure 2) In the C6 cell lines, sialic acid level was increased in the 9^{th} ($p<0.05$), 12^{th} and 15^{th} passages ($p<0.001$) when compared with the 3^{rd} passage.

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)
3^{rd} P	3	**0,14 ± 0,03**	**2,50 ± 0,09**
6^{th} P	3	**4,80 ± 1,26**	**7,40 ± 1,45**
9^{th} P	3	**0,62 ± 0,25**	**9,33 ± 4,03***
12^{th} P	3	**0,18 ± 0,09**	**17,40 ± 2,54*****
15^{th} P	3	**21,88 ± 5,53*****	**18,81 ± 0,32*****

Anova Test Sonucu: $F_{(4;10)}=27,33$ $p<0.001$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$)

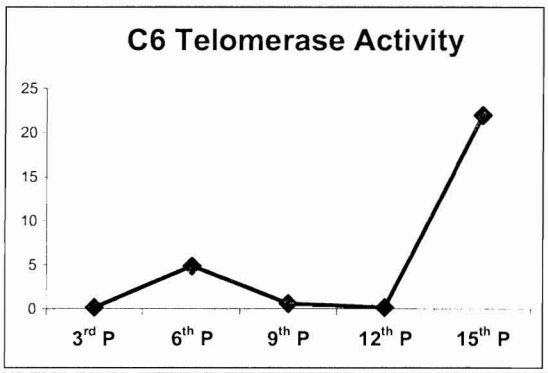

Figure 1

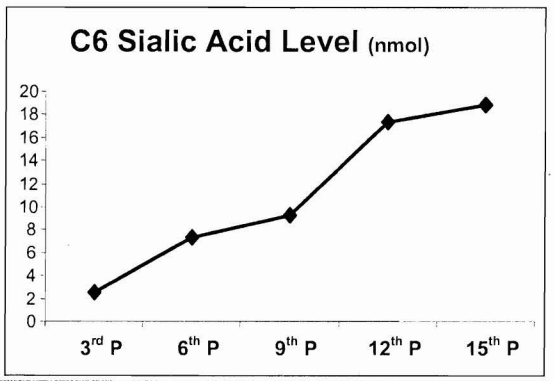

Figure 2

DISCUSSION

Telomerase Activity: The telomerase activity, generally positive in the cancer cells, increases in cell line with malignancy and aggressiveness (2,5,25-27). Telomerase activity has been manifested as positive in 11 (85%) of the 13 malign pancreas tumors, and as negative in 15 benign pancreas tumors (27). In accordance with these results, it has been claimed that the telomerase activity in pancreas tumors is an important parameter in determining the malignancy. 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 (28). 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 (29). 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. 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 (30). In 41 specimens with soft tissue tumors, it has been indicated that the telomerase positive cells were not only malignant but also aggressive (31). 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). 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 (32). 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 (33). 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.

Sialic Acid (NANA): 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 (34). 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 (35). 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 (36). 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 (37).

There was no suggestion showing how the level of sialic acid changes from passage to passage in our two cell lines. In the proceeding passages of the used cell lines, sialic acid level increased gradually, and this increase was more regular compared with the telomerase activity.

CONCLUSION

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 lines 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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