Antitumor Effects of Telomerase Inhibitor TMPyP4 in Osteosarcoma Cell Lines

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ABSTRACT INTRODUCTION:
The use of adjuvant and neoadjuvant chemotherapy has proved to be the effective treatment and plays an essential role in controlling osteosarcoma. Despite these advances, approximately, 30% of patients present with metastases and the prognosis for patients with osteosarcoma still is considered poor. In order to ensure a cure, it will be necessary to develop more effective adjuvant treatments. Telomeres are specialized structures containing unique (TTAGGG)n repeats at the ends of chromosomes which are thought to be important for the stabilization of chromosomes. Lagging strand DNA synthesis at the very end of chromosomes cannot be completed. This phenomenon is the so-called "end-replication problem" and this situation results in the progressive shortening of telomeric repeats with each cell division. Telomerase contains an RNA-dependent DNA polymerase, which compensates for the endreplication problem, and is expressed in germline cells but not in most somatic cells.

There are two telomere maintenance mechanisms in human tumors: telomerase activation and alternative lengthening of telomeres (ALT). Approximately 85% of all carcinomas engage a mechanism to maintain a stable telomere length by telomerase activity. ALT is characterized by a heterogeneous pattern of telomere length, usually ranging from very short to abnormally long, and a substantial proportion of sarcoma types have been reported to have elongated telomeres consistent with ALT in the absence of telomerase activity.

The telomere maintenance mechanism has recently reported as a prognostic factor for sarcomas, and ALT positivity correlates with survival of patients with several sarcomas. Therefore, studies of the telomere maintenance mechanisms may lead to novel therapeutic strategies to fight sarcomas.

Recent studies revealed that cationic porphyrin 5,10,15,20-tetra-(N-methyl-4-pyridyl) porphyrin (TMPyP4) can bind to DNA guanine-quadruplexes which are recognized in the single-strand G-rich overhang of telomeres and directly block telomerase elongation.

In order to examine the antitumor effects of telomerase inhibitor TMPyP4 in osteosarcoma cell lines, HOS (telomerase++, ALT-), MG63 (telomerase+, ALT+), SaOS2 (telomerase+, ALT+) and U2OS (telomerase-, ALT+) cells were treated with TMPyP4 after which cell growth, telomerase activity, telomere length were investigated.

METHODS:
Evaluation of telomerase activity was performed by fluorescein-based telomeric repeat amplification protocol (TRAP) assay. Telomere Length was analyzed with Southern blotting. Cell proliferation was examined by MTT assay. The cells were incubated in the presence of a TMPyP4 concentration of 1, 10, 50 or 100 &mu;M for 48h or 96h.

The significance of differences between groups was evaluated by the paired t-test. The level of significance was set at p < 0.05.

RESULTS SECTION:
1. TMPyP4 (10-100 &mu;M, 96h) inhibited in vitro telomerase activity in telomerase positive HOS cells (6% lower level compared with control values), MG63 cells (4%) and SaOS2 cells (16%).
2. Treatment with TMPyP4 for 96h significantly induced telomerase shortening in HOS cells (1 &mu;M: p = 0.011, 10 &mu;M: p = 0.029, 50 &mu;M: p = 0.0029, 100 &mu;M: p = 0.0039) and SaOS2 cells (10 &mu;M: p = 0.0039, 50 &mu;M: p = 0.0029, 100 &mu;M: p = 0.0029) (Figure 1). However, treatment with TMPyP4 did not induce telomerase shortening in U2OS cells.
3. Treatment with TMPyP4 at doses of 50 &mu;M for 48h or 96h significantly inhibited the growth of HOS cells (48h: p = 0.0045, 96h: p = 0.00011) (Figure 2), SaOS2 cells (48h: p = 0.0001) (Figure 2), and U2OS cells (48h: p = 0.016, 96h: p = 0.0003) (Figure 4) but not in MG63 cells (96h: p = 0.11).

DISCUSSION:
G-quadruplex-interactive agents TMPyP4 reported to be a telomerase inhibitor require long-term culture to show telomerase inhibition and telomere shortening. In our study, TMPyP4 inhibited telomerase activity in telomerase positive cells. However, the down levels of telomerase activity ranged only 4 to 16%. Telomerase activation may be prevalent in several osteosarcoma cell lines, although even lower activity levels played a role. G-quadruplex-interactive agents may not be able to inhibit sufficient levels of telomerase activity in osteosarcoma cells.

Treatment with TMPyP4 significantly induced telomere shortening in HOS cells and SaOS2 cells. In addition, treatment with TMPyP4 significantly inhibited the growth of HOS cells and SaOS2 cells. In many cells, when telomeres become critically short, further cell division is blocked, a process often referred to as replicative senescence. Telomere shortening by TMPyP4 may inhibit cell growth in HOS and SaOS2 cells.

Treatment with TMPyP4 did not induced telomere shortening but significantly inhibited the growth of U2OS cells. The antitumor effect of TMPyP4 for some osteosarcoma cells may be related to DNA damage including telomere dysfunction through G-quadruplex stabilization, independent on telomere length.

Sarcoma cells have a relatively low telomerase expression compared to carcinoma cells. In addition, several types of sarcoma have ALT and exhibit a remarkable elongated telomere length, indicating telomerase inhibitor may not be effective theoretically. However, our results may indicate telomere maintenance mechanisms are novel targets of adjuvant therapy for osteosarcoma patients. Further study is necessary to clarify the mechanisms of antitumor activity by TMPyP4.

Figure 1: Telomere lengths of SaOS2 cells
Figure 2: MTT assay of HOS cells
Figure 3: MTT assay of SaOS2 cells
Figure 4: MTT assay of U2OS cells