Student Name:  

Please begin research abstract here: (Include title, hypothesis, specific aim(s), rationale and general research methods). Be specific as to what you will be doing and the extent of your own participation in the proposed research. No more than 2 pages (References only can extend onto a 3rd page).

Telomerase inhibition in canine osteosarcoma using a G-quadruplex inhibitor

Student: XXX

Faculty mentor: XXX

Hypothesis

We hypothesize that by inhibiting telomerase in canine osteosarcoma we will reduce telomere length and consequently decrease cellular proliferation and clonogenicity.

Specific Aims:

Specific Aim 1: Determine the effect that TMPyP4 has on telomerase activity in canine osteosarcoma.

Specific Aim 2: Determine the effect that TMPyP4 has on telomere length in canine osteosarcoma.

Specific Aim 3: Measure the effects on cellular proliferation and clonogenicity in cells treated with TMPyP4.

Rationale:

Osteosarcoma is an aggressive cancer and the most common primary bone tumor in dogs, accounting for up to 85% of skeletal malignancies and leading to death due to metastasis in up to 72% of dogs within two years of diagnosis even with aggressive treatment [1]. Current standard of care therapies include limb amputation or limb sparing surgery in conjunction with chemotherapy or radiation therapy but despite these treatments osteosarcoma prognosis remains poor [2, 3].

Cancer cells can bypass a variety of cellular checkpoints avoiding cellular senescence and apoptosis while undergoing excessive proliferation, thus forming tumors. Telomeres are tandem repeats of the sequence TTAGGG located at the ends of chromosomes and are well conserved between species [4, 5]. As chromosomes are replicated in the normal mitotic cell cycle, DNA polymerases fail to replicate the entirety of the telomere at the end of the chromosome and therefore cause telomeres to shorten and the cell to undergo apoptosis when telomeres shorten to a critical point after multiple cycles of replication [6, 7]. The telomerase enzyme is one of the main cancer cellular mechanisms used to maintain telomere length by adding telomeric repeats and therefore avoiding senescence or cell death [8].

Located on telomeres are structures known as G-quadruplexes that, when stabilized in human cancer cells, have been shown to inhibit telomerase activity, therefore decreasing telomere length and slowing cell proliferation [9-11]. In human cancers, including osteosarcoma [9], a drug called TMPyP4 has been shown to act as an anti-telomerase agent by acting not on telomerase itself, but through stabilization of G-quadruplexes. Importantly, TMPyP4 appears to remain nontoxic to both cancer and non-cancer cells [10].

The biologic behavior of human and canine osteosarcomas is reported to be remarkably similar and telomerase activity has been identified in up to 95% of canine cancers including osteosarcoma [12-14]. Because both telomeres and telomerase are so highly conserved between species, it is likely that the G-quadruplex structure that has been reported in human lines will be present in canine osteosarcomas. Therefore it is hypothesized that the G-quadruplex stabilizing drug TMPyP4 will work to lower telomere activity and consequently decrease cellular proliferation in canine osteosarcoma cells.

General research plan:

Several methods can be used to measure the efficacy of TMPyP4 as an anti-cancer agent at a variety of locations in the cancer lifecycle. Because TMPyP4 acts as a telomerase inhibitor, our first aim is to measure telomerase activity in osteosarcoma cells treated with this drug as compared to control osteosarcoma cells. Telomerase inhibitors work through telomere-shortening mechanisms, therefore our second aim is to measure telomere length and compare that to the length of telomeres in osteosarcoma cells that are not treated with this drug. Lastly, the ultimate goal of TYMPyP4 as an anti-cancer drug is to decrease the rate of cancer cell proliferation, so our final aim is to measure the rate of proliferation in the treated and control lines. We expect to see decreased telomerase activity, shortened telomeres, and decreased cell proliferation in osteosarcoma cells.
Three canine osteosarcoma cell lines which were derived from naturally developing tumors in clinical patients from our veterinary medical teaching hospital will be used in these experiments. The development and characterization of one of these cell lines, as well as the authentication method used has been previously published [15, 16]. Cells will be maintained in Dulbecco’s modified Eagle medium high glucose with L-glutamine and sodium pyruvate and supplemented with 10% heat-inactivated fetal bovine serum and 100 U of penicillin-streptomycin/mL. Cells will be grown in T-75 flasks at 37°C in a humidified environment with 5% carbon dioxide and 95% air. Each of the experiments will be done with each cell line in triplicate.

Specific Aim 1: TRAPEze XL telomerase detection kit will be used to measure telomerase activity. Cells will be plated into a 96 well plate and will be left untreated or treated with 50 μM or 100 μM TMPyP4. Cells will be assayed according to manufacturer instructions to determine telomerase activity after 24, 48, 72, and 96 hours of incubation 37°C in 5% CO2. The experiment will be repeated in triplicate.

Specific Aim 2: TeloTAGGG telomere length assay will be used to measure telomere length. Cells will be plated into a 96 well plate and will be left untreated or treated with 50 μM or 100 μM TMPyP4. Cells will be assayed according to manufacturer instructions to determine telomerase activity after 24, 48, 72, and 96 hours of incubation 37°C in 5% CO2. The experiment will be repeated in triplicate.

Specific Aim 3: Growth curve analysis will be done to assess cellular proliferation. Cells will be plated into a 96 well plate and will be left untreated or treated with 50 μM or 100 μM TMPyP4. Cells will be counted to determine the concentration of cells using a Coulter counter after 24, 48, 72, and 96 hours of incubation 37°C in 5% CO2. Cells from tissue culture in logarithmic growth phase (70% to 80% confluent) will be trypsonized and counted and then transferred to 60-mm Petri dishes (initial density, 500 cells/plate) and returned to the aforementioned growth conditions. Cells will be left untreated or treated with TMPyP4. Plating efficiency will be determined by removing medium after 7 days of culture and staining cell colonies with crystal violet so that colonies can be counted. Only colonies of > 50 cells will be counted manually and the surviving fraction calculated. Each of the experiments will be repeated in triplicate.

Statistical Analysis:

All data collected will be continuous data and means and standard deviations will be determined. Data will then be checked for normality. If the data is normal t-tests will be done to look for differences between the treated cells and controls for each cell line. If the data is not normal non-parametric analysis will be done.

Potential Pitfalls

It is possible that telomerase activity does not play a role in the cell lines we are using as this has not been tested. It is an unlikely finding as telomerase activity has been demonstrated in canine osteosarcoma by other investigators; if this were found however, it would be an interesting result in itself. It is also possible that the G-quadruplex structure is not formed in canine cells as this has not been evaluated in dogs. It is unlikely given the highly preserved nature of this structure and canine telomeres are of the same sequence as other species. Again this would also be an interesting finding if dogs did not form this structure.
References