

Palovarotene, the Selective Agonist for Nuclear Retinoic Acid Receptor Gamma, Inhibits Proteoglycan Production and Decreases Tumor Mass Size in a Human Chondrosarcoma Cell Line

William Shield¹, Yang Dan¹, Ashley Cellini¹, Masaharu Takigawa², Masahiro Iwamoto¹, Motomi Enomoto-Iwamoto¹ and Vincent Ng¹
¹University of Maryland, Baltimore, MD, USA, ²Okayama University, Japan

Objectives

Background

Chondrosarcoma (CS) is the most common primary skeletal sarcoma in adults. Treatment of CS is currently limited to surgery. An ideal therapy for CS would be a minimally-invasive local or systemic treatment modality to halt growth within the lesion and kill the tumor cells. At present this is a far reaching goal. Previous research on retinoic acid (RA) has demonstrated that this molecule alters chondrocyte growth *in vitro* and can cause regression of CS in animal studies, but RA has potential to cause severe adverse effects. Palovarotene, an agonist of nuclear retinoic acid receptor gamma (RAR γ), has been studied in a Phase-III human clinical trial for treatment of heterotopic ossification in patients with fibrodysplasia ossificans progressiva without reports of critical side effects (NCT03312634). Furthermore, a clinical trial on Palovarotene has been started for Multiple Osteochondroma (NCT03442985). These prior findings as well as the effects of Palovarotene on the biology of human cartilage lead us to the idea that Palovarotene and other RAR γ agonists may be applicable to chondrosarcoma. We hypothesized that human CS specimens retain RAR γ signaling and that both systemic and local administration of palovarotene results in decreased growth in an *in vitro* and *in vivo* animal model.

Objectives

The aims of our study were (1) to determine if RAR γ is expressed in human chondrosarcoma specimens and (2) to determine the *in vitro* and *in vivo* therapeutic action of selective RAR γ agonists in a human CS cell line.

Materials and Methods

Human CS specimen staining: Human CS specimens were immunostained with anti-RAR γ antibody (Sigma).

Cell culture and *in vitro* treatment: A human CS cell line (HCS2.8) established from grade II chondrosarcoma (72yr, male) was used.

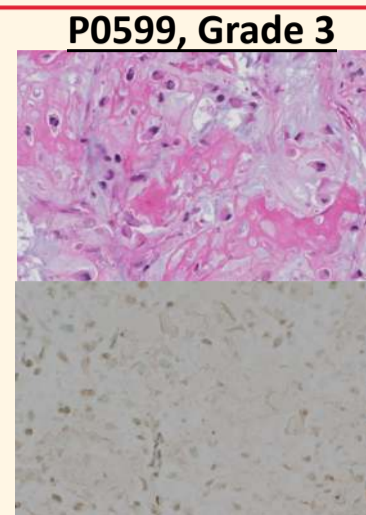
Systemic and local *in vivo* Palovarotene treatment: Cells (1 million cells in 100ml) were inoculated subcutaneously in NOD/Shi-scid IL2r γ (null) (NOG) mice. For systemic treatment, mice were treated with Palovarotene (5 mg/kg) or the same volume of vehicle (1:9, DMSO:corn oil) via gavage 3 times/week starting 1 week after tumor inoculation demonstrated a viable cell mass (n=10). Local treatment was performed with subcutaneous injections (every 3-4 days) of nanoparticles tagged with Palovarotene (5 mg/kg) or the same volume of untagged nanoparticles (n=5).

Summary and Conclusion

- All human CS specimens expressed RAR γ .
- Palovarotene treatment reduced the mass size of pellet cultures (HCS2/8 human chondrosarcoma cell line cells).
- HCS2/8 cells responded to RAR γ agonists and reduced tumor mass size when subcutaneously implanted in mice. This finding was more pronounced when nanoparticle drug delivery was utilized.
- To our knowledge, this is the first evaluation of effects of Palovarotene on a human chondrosarcoma cell line. Future studies will focus on multiple cell lines and the effect of Palovarotene not only on primary tumor growth but also on metastasis.

Results

Figure 1. Human chondrosarcomas retain RAR γ expression.



Number	Grade	Staining
P0593	Grade 3	+
P0594	Grade 3	+
P0595	Grade 1	++, strong in nuclei
P0596	Grade 2	+
P0597	Grade 1	++, strong in nuclei
P0598	Grade 2	+, some are negative
P0599	Grade 1	++, strong in nuclei, variation

Figure 2. Palovarotene decreased GAG and protein content and reduced cartilage matrix gene expression in HCS2/8 cells *in vitro*.

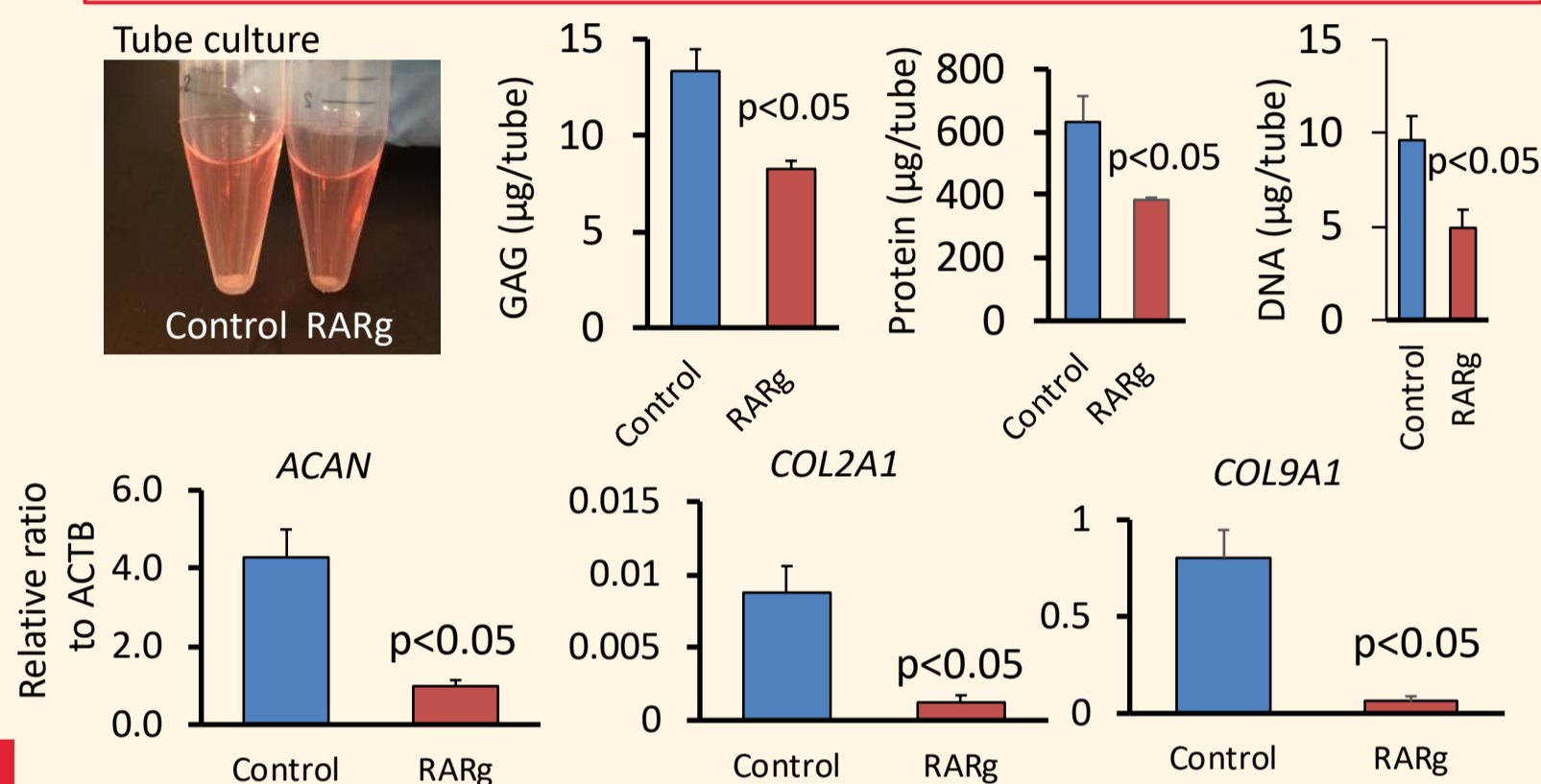
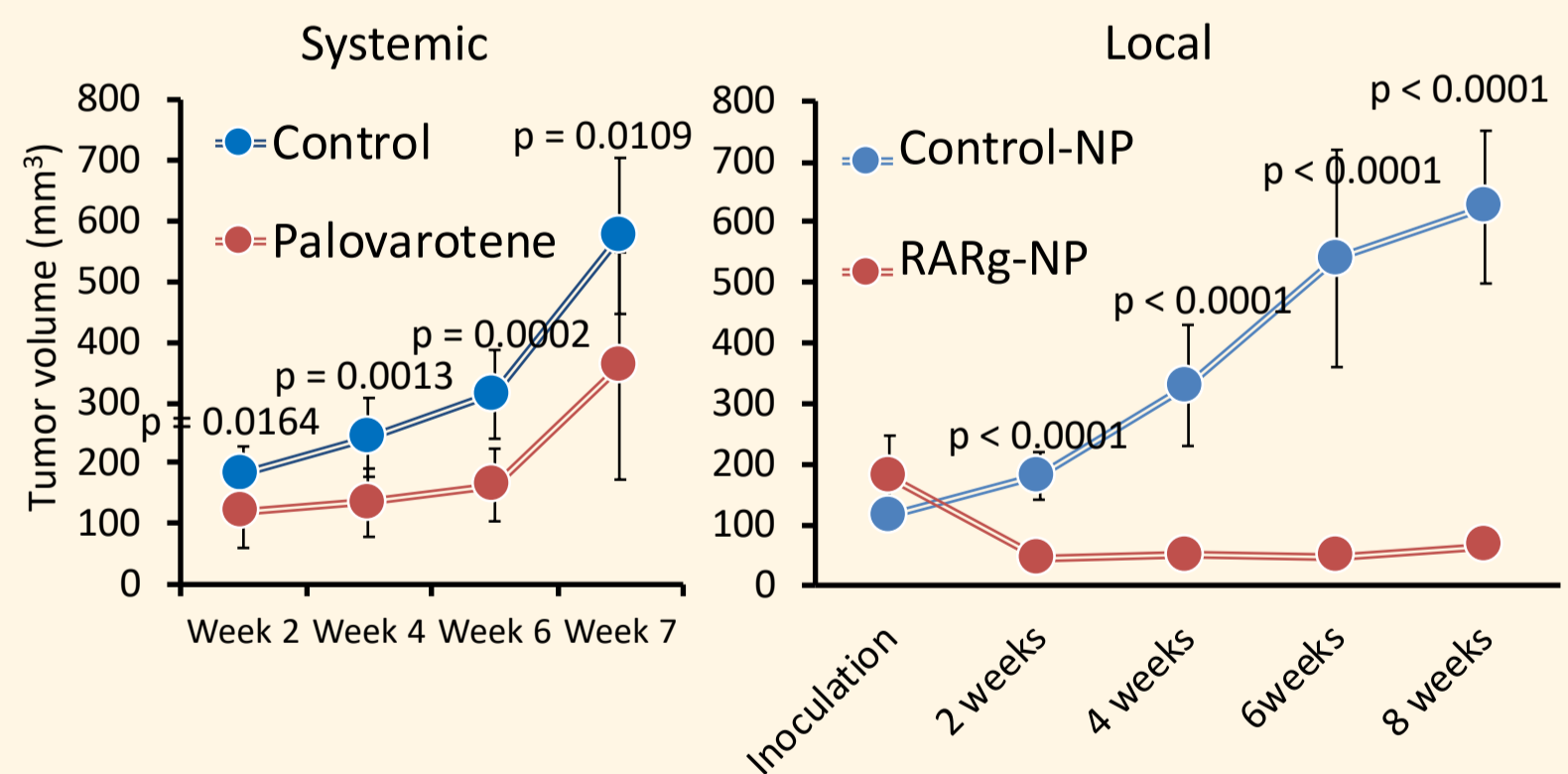


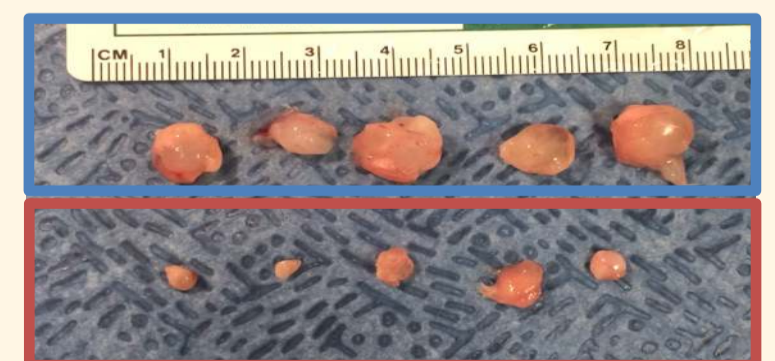
Figure 3. Palovarotene inhibited tumor growth, and RAR γ -nanoparticles reduced tumor mass and stimulated surrounding fibrous connective tissue.



Gross appearance

Control-NP

RAR γ -NP



Histology

Control-NP

RAR γ -NP

