DKK-1 in Osteosarcoma: targeting dual mechanisms

Simin Pan*, Andrew Haskell†, Michael Cesarek±, Kelbi Padilla‡, Carl A. Gregory

* Department of Molecular and Cellular Medicine, Institute for Regenerative Medicine, Texas A&M Health Science Center College of Medicine
† Department of Microbial Pathogenesis and Immunology , Texas A&M Health Science Center College of Medicine
± *Biology major,* Texas A&M University
‡ Biomedical Sciences major, Texas A&M University

HEALTH SCIENCE CENTER
TEXAS A&M UNIVERSITY

Introduction

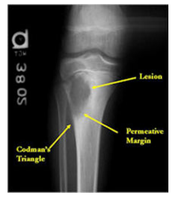


Figure. 1

Malignant bone disease (MBD) is characterized in part by osteolytic lesions (OLs), defined as tumor-filled holes in bone tissue **[Fig1]**. OLs frequently fail to heal, even with interventions, providing an effective niche for tumor repopulation. OLs also cause untreatable pain and fracture.

It is known that tumors secrete Wnt inhibitors (WIs), which have the capacity to inhibit canonical Wnt (cWnt) signaling, a key pathway that drives the differentiation of bone marrow mesenchymal stem cells (MSCs) to bone-synthesizing osteoblasts.

Several types of WI are involved in OL formation, but Dickkopf-1 (Dkk-1) is the most common, associated with multiple myeloma (MM), osteosarcoma (OS) and breast and prostate metastases.

High serum DKK1 gynecological cancer, multiple myeloma, Hepatocellular Carcinoma, breast cancer are also related to poor prognosis in patient.

Our goal is to modulate the activity of WIs (most notable Dkk-1) to prevent or repair OLs, and possibly inhibit disease progression.

Previous Work

Our lab found that recombinant expression of human Dkk-1 in a murine osteochondral tumor cell line (MOSJ-DKK-1 cells) profoundly increased the rate of tumor growth as well as bone destruction in an orthotopic mouse model.

The results further suggested that the enhanced tumorigenesis was caused by up-regulation of aldehyde dehydrogenase 1A1 (ALDH1A1) through a mechanism involving a novel, non-canonical Wnt (ncWnt) pathway. We found that when Dkk-1 inhibited the osteogenic canonical Wnt (cWnt) pathway, the novel ncWnt pathway predominated, resulting ALDH1A1 upregulation, proliferative enhancement and resistance to metabolic chemotherapeutic stresses **[Fig2],** (Krause *et al. 2014, Cell Death Dis.*)

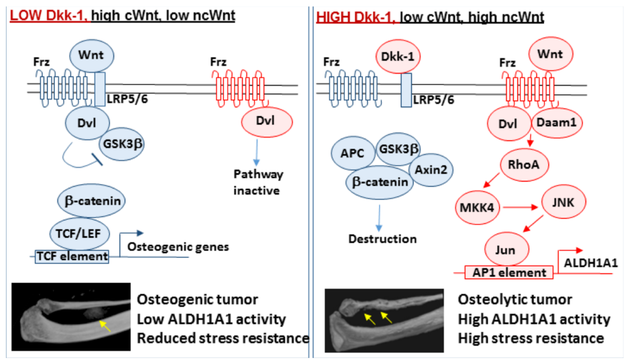


Figure. 2

Targeting dual mechanisms

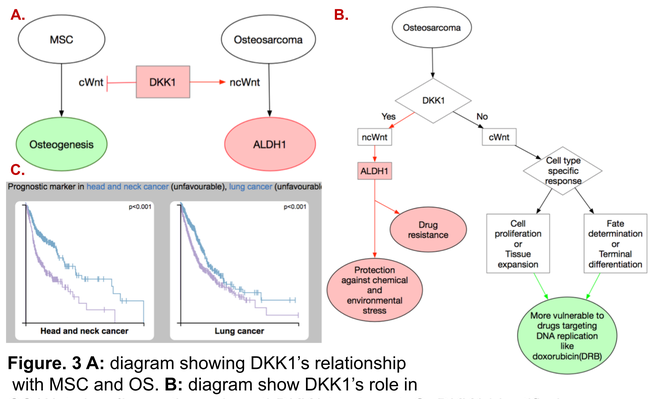


Figure. 3 A: diagram showing DKK1's relationship with MSC and OS. **B:** diagram show DKK1's role in OS Wnt signaling pathway in anti-DKK1 treatment. **C:** DKK1 identified as unfavorable prognostic genes in different cancer (Uhlen, Mathias, et al. 2017, Science)

In vitro proof of concept.

DRB dose response *in vitro* with MOSJ-Dkk1 cells. To ascertain the range of effective DRB doses, and thereby estimate the sub-clinical dose of DRB, a dose response study was performed. **[Fig4A. B].**

Vivo-Morpholinos dose response *in vitro*. Using MOSJ-DKK-1 cells *in vitro*, we demonstrate that Dkk-1 blockade is achievable using vivo-morpholinos **[Fig4C].**

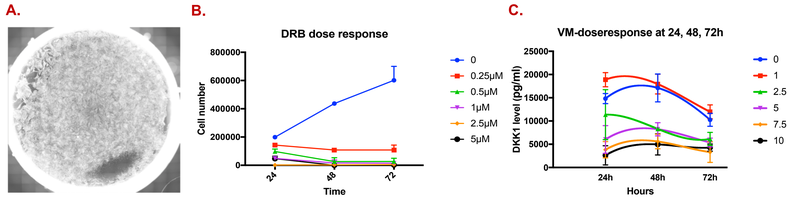


Figure. 4 A: Example image of one well at 40x. **B:** There is significant evidence(α=0.10) that cell counts are different across 0, 0.25, 0.5, 1, 2.5 and 5 μM range at 24h, 48h and 72h. (AOV at 0.05, Kruskal-Wallis Test at 0.10 the difference is most likely due to low replication number). **C:** There is significant evidence (α = 0.05, FDR = 0.05) that vivo-morpholinos targeting human DKK-1 sequence lower the medium human DKK-1 level in MOSJ-DKK cell *in vitro* at 5, 7.5 and 10 μM at 24h, 48h and 72h

Ongoing studies *in vivo.*

We utilize vivo-morpholino technology to block Dkk-1 expression in mice implanted with MOSJ-Dkk1 cells. After 14 days, we will measure Dkk-1 expression, ALDH1A1 expression, tumor expansion, and bone destruction. In further experiments, we will also administer DRB with anti-Dkk1 vivo-morpholinos to assess whether Dkk-1 blockade enhances sensitivity to DRB. We dosed dsRed labeled MOSJ-Dkk1 harboring mice with vivo-morpholinos, 1 and 5 mg/kg DRB for 2 weeks and imaged them with an Ivis Lumina live animal imager **[Fig5]**.

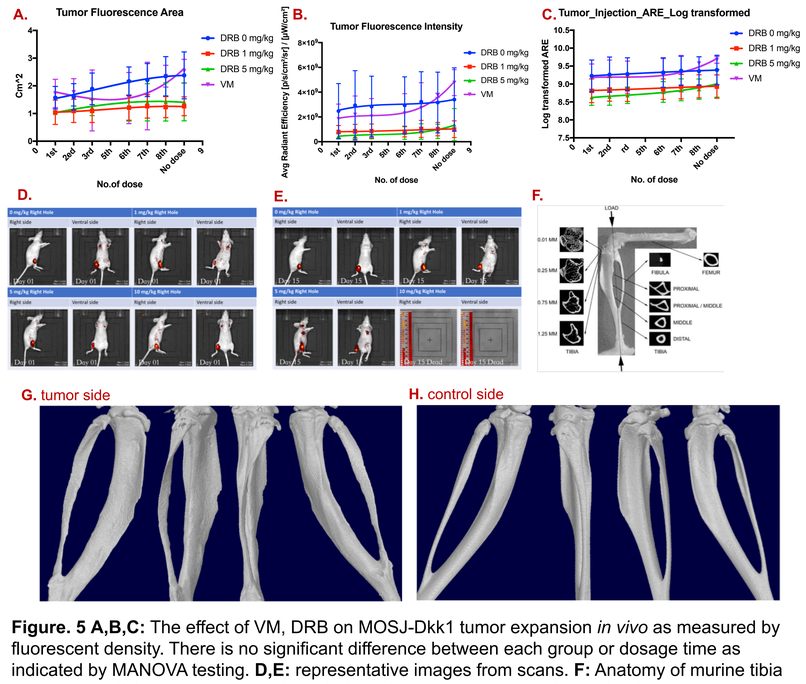


Figure. 5 A,B,C: The effect of VM, DRB on MOSJ-Dkk1 tumor expansion *in vivo* as measured by fluorescent density. There is no significant difference between each group or dosage time as indicated by MANOVA testing. **D,E:** representative images from scans. **F:** Anatomy of murine tibia and fibula. MOS-J cells were injected into the interosseous space between the tibia and fibula, which is more clinically relevant. (Sugiyama, et al 2010 *Bone)*. **G, H:** μCT scan for bone disruption of tumor and control side. (scanned under 12.7 μm resolution)

In contrast with the in vitro testing, we found that VM or DRB alone had no significant effect on MOSJ-Dkk1 tumor size *in vivo* (although a slight trend was observed for some imaging protocols) **[e.g. Fig5C].**
We are planning to move forward with co-administrations of VM and DRB.

These data demonstrate the disparity between monolayer culture experiments and in vivo testing with respect to chemotherapy drug efficacy. It is also possible that Dkk-1 expression results in enhanced drug resistance *in vivo*.

Knock down of Dkk-1 in human cells.

To define how Dkk-1 upregulates ALDH1A1 expression in human bone tumor cell lines and to examine how this affects resistance to hypoxia and other stresses in cell culture are generating SAOS, MG63, U226 and INA6 bone tumor cells with stable knock down of Dkk-1 expression. All express inherently high levels of Dkk-1. We use lentivirally transduce the cell lines with short hairpin RNA (shRNA) directed against human Dkk-1 and the knock down **[Fig6.A]** cell lines marked with td-Tomato.

Effect of proliferation and chemical stressors are tested. SAOS and MG63 5, 10 and 15 days of monolayer growth, doxorubicin (DRB, 0.25 μM) or hydrogen peroxide (100 μM) for 48 hr. DKK1, ALDH1A1 and JNK and p-JNK were determined **[Fig6.B,C.]**

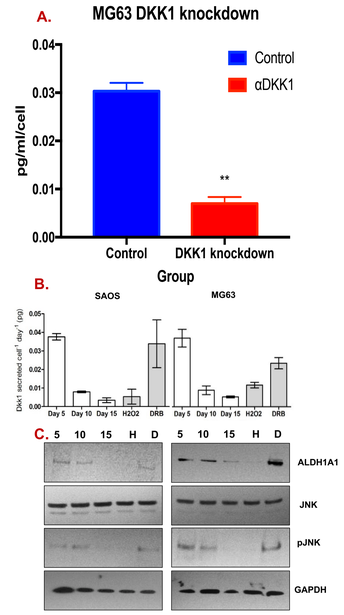
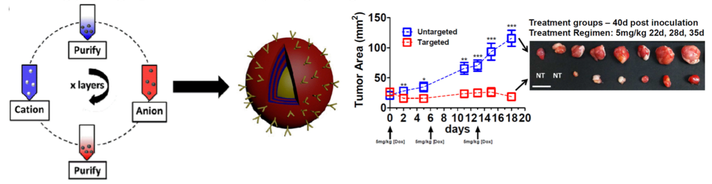


Figure 6. A: MG63 DKK1 knock down normalized to cell number. **B:** Dkk-1 level normalized to cell number. **C:** Immunoblots for ALDH1A1, JNK and p-JNK the various conditions.

Next Steps…

i) To examine whether co-administration of knock down Dkk-1 morpholinos and DRB synergize in the MOSJ-Dkk1 model of osteosarcoma.

ii) Examine alternative approaches for Dkk-1 knock down *in vivo*. Test a novel non-viral gene modulation strategy **[Fig7]** and antibody targeting.



Morton, Stephen W., et al. "Osteotropic Therapy via Targeted Layer-by-Layer Nanoparticles." *Advanced healthcare materials* 3.6 (2014): 867-875.
Quadir, Mohiuddin A., et al. "Ligand-decorated click polypeptide derived nanoparticles for targeted drug delivery applications." *Nanomedicine: Nanotechnology, Biology and Medicine* (2017).

Figure. 7

iii) Address contrast between DRB activity *in vitro* and *in vivo*. Our data reveal the gap between *in vitro* and *in vivo test result*. We will use a novel 3 dimensional bone/tumor co-culture system **[Fig8]** to test approaches for blockade of Dkk-1, ALDHA1 and other targets *in vitro*.

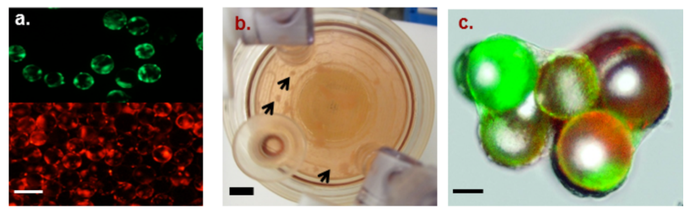


Figure. 8 A:'GFP;labeled" hMSCs" (upper)" and" dsRed;" labeled" MOSJ;Dkk1" cells" (lower)" on" micro;spheres" after" RWV" culture" (bar" =" 150" μm)." **B:**" RWV" containing" aggregated" cell;laden" microspheres" (arrowed)" (bar" =" 10" mm)." **C:**' Bead" cluster" with" green," red" and" mixed" micro; spheres" (bar" =" 75" μm).'

Acknowledgements:

Gregory's lab members: Suzanne Zeitouni, Eoin Mc Neill
Students: Michael Cesarek, Daniel Tahan
Department of Molecular and Cellular Medicine, Institute for Regenerative Medicine, Texas A&M Health Science Center College of Medicine.
This work is funded by a High Impact High Return (CAG) and an Investigator Initiated Award (CAG) from the Cancer Prevention and Research Institute of Texas (CPRIT).