Reduced Sirtuin 1, the Anti Aging Gene, at the Femoral Neck Inferior Medial Cortex (Calcar Region) in Osteoporotic Hip Fracture

Madi El-Haj, MD¹, einav Cohen-Kfir, Ph.D.¹, Hanna Artsi¹, Irina Gurt, Ph.D.¹, Ori Safran, MD¹, Rivka Dresner-Pollak, MD². ¹Hadassah Hebrew University Medical Center, Jerusalem, Israel, ²Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Disclosures:

Introduction:
Bone loss and osteoporosis are inevitable consequences of aging. Recent advances in aging research have identified the sirtuins as major players in aging processes and in age-associated diseases. Sirtuin 1 (Sirt1), the most investigated sirtuin, was shown to play a role in diabetes, neuro-degeneration and cardiovascular disease. We were the first to report that Sirt1 regulates bone mass in female mice (Cohen-Kfir Endocrinology 2011; 152:4515-24). Importantly, we identified sost, encoding for sclerostin, an important bone formation inhibitor, as a novel target of Sirt1, and demonstrated that Sirt1 inhibits sost expression by deacetylating histone 3 lysine 9 at its promoter. The importance of sclerostin in humans was first appreciated when excessive bone mass was observed in patients with mutations or deletions in the sost gene (Van Buchen’s disease or sclerostosis, respectively). Moreover, increased serum sclerostin was identified as an independent risk factor for hip fractures in elderly women, and an anti sclerostin neutralizing antibody is an emerging novel therapy for osteoporosis. Thus, modulating sclerostin by means such as Sirt1 activation is a potential novel approach to design new anabolic therapies for osteoporosis.

Data regarding local expression of Sirt1 and sclerostin in patients who sustained a femoral neck hip fracture are lacking, and may contribute to our understanding of the role of Sirt1 in osteoporosis and in osteoporotic hip fractures.

The goal of this study was to investigate the relationship between protein level of Sirt1 and sclerostin at the various femoral neck regions in women who sustained a hip fracture.

Methods:
Elderly women (age>60 years) who were admitted to Hadassah-Hebrew University Medical Center, Jerusalem, Israel with a subcapital femoral neck fracture requiring partial hip replacement were recruited for this study. All participants signed informed consent. Exclusion criteria included conditions affecting bone mineral metabolism (renal failure, diabetes, thyroid and parathyroid disorders, malignant conditions, previous orthopedic surgery at the affected side, use of anti-osteoporotic medications or hormone replacement therapy). The study was approved by the institutional ethical committee HMO-36106. Bone samples from four different femoral neck regions were collected intra-operatively: two cortical bone samples (calcar and superior lateral cortex), two trabecular bone samples (subchondral and intramedullary) (Figure 1A). Samples were cleaned of soft tissue and periostium, dissected into bone chips and immediately snapped freeze in liquid nitrogen. All samples were kept in -80°C pending protein extraction.

Protein was extracted from 15mg of bone sample. Samples were crushed, extensively washed in PBS, immersed in sample buffer (10% Glycerol, 2% SDS, 60mM Tris pH=6.8, 2% 2-mercaptoethanol). Protein quantification was conducted with the RC/DC protein assay (BIO-RAD). Equal amounts of protein were separated by SDS-PAGE electrophoresis. The following antibodies were used for
immuno-blots: anti Sirt1 (Upstate-Millipore), anti sclerostin (Abcam), anti H2B (Cell Signaling). Protein expression was detected using Z-ECLWB detection kit (Beit Haemek). The relative densities of protein bands were analyzed using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA), calibrated to the density of the house keeping gene H2B, and normalized to the superior lateral cortex region. Five osteoporotic women were included in this study.

**Results:**
A significantly lower level of Sirt1 was found in the inferior medial-calcar region, the area subjected to the highest compressive loads, compared to the superior lateral cortex region (Figure 1B). On the other hand, the highest sclerostin level was detected in that same area- the calcar region (Figure 1C).

Importantly, sclerostin and Sirt1 were inversely expressed (Figure 1D), supporting the hypothesis that Sirt1 is a negative regulator of sclerostin. Figure 1: A- Schematic illustration of human femur indicating bone samples harvesting sites: CI- calcar inferior medial, Sub.Tb-subchondral trabecular bone (subcapital), SC- superiorlateral cortex, Intra.Tb-intramedullary trabecular bone. B- Sirt1 and C- Sclerostin protein levels at the 4 regions determined by western blot analysis. A representative image is presented. D- Densitometry of western blot analyses of SIRT1 and sclerostin relative to H2B obtained in 6 patients. Data are represented as Mean ± SEM normalized to SC region. Statistical analysis was performed by Student’s t test *P<0.05

**Discussion:**
Regional differences and reduced Sirt1 protein level at the calcar region of the femoral neck in female patients who sustained an osteoporotic hip fracture may implicate a role for Sirt1 in osteoporosis, and set the stage to investigate the role of recently generated sirtuin 1 activators for the treatment of osteoporosis. Furthermore, the bone anabolic potential of Sirt1 activation by sclerostin modulation or other mechanisms remains to be unraveled.

**Significance:**
Based on our previously published data (Cohen-Kfir *Endocrinology* 2011; 152:4515-24) demonstrating that Sirt1 inhibits sclerostin, and the above human study, we hypothesize that specific SIRT1 activators may provide a novel strategy for generating new anabolic therapies for osteoporosis.

**Acknowledgments:**

**References:**
ORS 2014 Annual Meeting
Poster No: 1428