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Full Length Article

Validation of a novel, rapid, high precision sclerostin assay not confounded by sclerostin fragments

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ABSTRACT

Sclerostin is a 190 amino acid protein secreted primarily by osteocytes. It was initially identified due to mutations in the SOST gene associated with high bone mass phenotypes. Much recent work has sought to determine the importance of sclerostin across an array of conditions which affect the human skeleton. However, accurate measurement of sclerostin from serum and plasma sources remains a significant impediment, with currently available commercial assays showing marked differences in measured sclerostin values. Accordingly, sclerostin assay standardization remains an important but unmet need before sclerostin measurements can be used for the clinical management of bone disease. Here we characterize a novel automated chemiluminescent sclerostin assay (LIAISON®, DiaSorin) which overcomes many of these limitations. Important assay characteristics include: a wide dynamic range (50–6500 pg/mL); high intra- (<2.5%) and inter- (<5%) assay precision; matched serum and plasma equivalence (<10% difference); specificity for the intact sclerostin molecule; and rapid assay results. Serum sclerostin levels measured with the LIAISON® assay in a population-based sample of adult men (n = 278) and women (n = 348) demonstrated that sclerostin levels were significantly higher in men as compared to women and were positively associated with age in both sexes, consistent with previously published work. In postmenopausal women, serum sclerostin levels measured with the LIAISON® assay were reduced in response to treatment with either estrogen or teriparatide, again consistent with previous findings. Collectively, the above data demonstrate that the LIAISON® sclerostin assay provides a reliable tool for more confident assessment of emergent mechanisms wherein sclerostin may impact a number of bone related pathologies.

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1. Introduction

Sclerostin is a soluble glycoprotein secreted primarily by osteocytes [1]. Mutations in SOST, the gene encoding sclerostin, have been identified as the causative defect in patients with the rare high bone mass disorder sclerosteosis [2,3]. Following these initial findings, understanding of sclerostin biology has evolved dramatically. The canonical osteoanabolic Wnt/β-catenin pathway underlies nearly all facets of osteoblast biology including osteoblast differentiation, proliferation, survival, and ultimately activity. There is now good evidence that sclerostin serves as a central mediator of skeletal anabolism due to its function as an endogenous soluble antagonist of Wnt/β-catenin signaling.

Despite recognition of sclerostin's integral role in skeletal metabolism, significant questions remain about the function of sclerostin both in normal skeletal physiology and in various pathologic conditions which affect the skeleton. Some of this uncertainty regarding the utility of circulating sclerostin as a biomarker reflects current deficits in our knowledge of normal biologic variables that likely effect sclerostin levels, including whether circadian or seasonal effects impact sclerostin levels, the mechanism by which sclerostin is cleared from the circulation, and the relationship between total body bone mineral content and sclerostin levels. An additional source of uncertainty reflects the belief that sclerostin functions primarily locally to integrate paracrine and possibly autocrine factors, although recent evidence indicates that circulating sclerostin may regulate peripheral fat depots and therefore function as a systemic hormone [4]. Nearly all reports in humans have examined circulating (serum and plasma) sclerostin levels in efforts to better understand the role of sclerostin in human biology. In support of this approach, there is evidence in humans that circulating sclerostin levels are highly correlated with bone marrow plasma sclerostin levels [5]. Given this strong correlation, it appears appropriate to consider circulating sclerostin levels as a reasonable surrogate for sclerostin levels

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in the bone microenvironment, keeping in mind that particularly the intact molecule may also have systemic hormonal effects [4].

To this end, several commercial immunoassays have been developed. To date, three have dominated the published literature. These include an ELISA from BioMedica (the most commonly reported assay), an ELISA from TECO*medical*, and an electrochemiluminescence immunoassay produced by Meso Scale Discovery. More recently, an ELISA from R&D Systems was described [6]. However, while accurate measurement of sclerostin levels may ultimately be of significant value for the diagnosis of disorders of bone modeling and remodeling, including the response to therapeutic intervention, substantial discordance in reported sclerostin values between the various commercially available assays currently precludes the ability to compare results across studies [6–11], and confounds understanding of the clinical implications of sclerostin value measurement.

Here we describe a novel, rapid sclerostin assay (hereafter referred to as LIAISON sclerostin CLIA) which overcomes many limitations inherent to earlier assays, including specifically both the lack of matched serum and plasma equivalence and the measurement of sclerostin fragments in addition to intact sclerostin. Assay validation was performed in an age-stratified adult population-based sample, as well as in direct human interventional studies in which circulating sclerostin levels were shown to decline in response to treatment with estrogen and teriparatide.

2. Materials and methods

2.1. Methods

The LIAISON Sclerostin CLIA (Fig. 1A) is a recently developed assay from DiaSorin. The first incubation step (10 min) allows for the

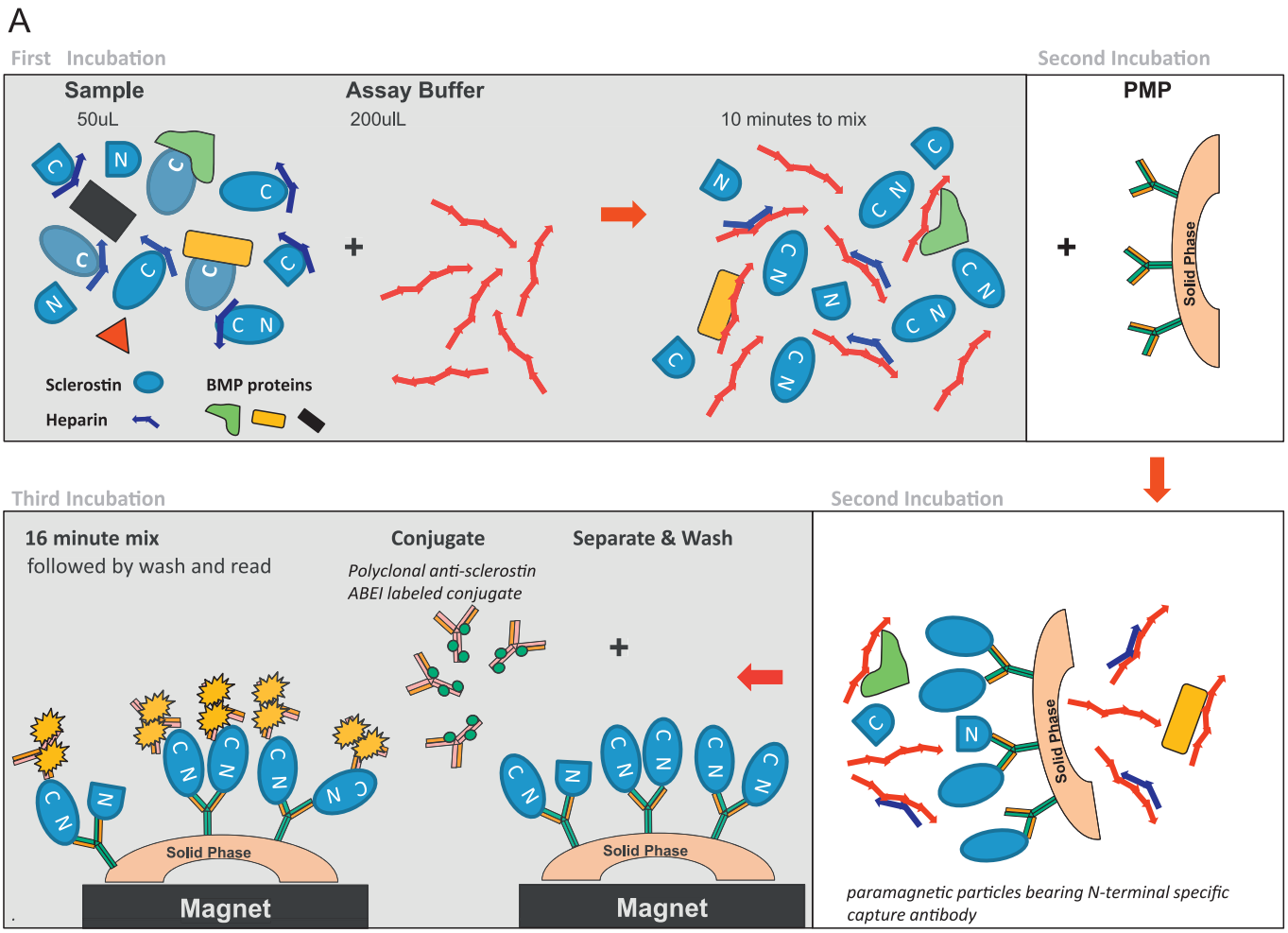

Fig. 1. A. LIAISON sclerostin CLIA. First incubation 1: 50 μl patient sample incubated with an assay buffer containing a basic polymer to dislodge anionic binding partners like heparin and BMP proteins than can occlude sclerostin's C-terminal surface, making it more accessible to conjugate recognition. Second incubation: N-terminal specific murine monoclonal capture antibody coupled to paramagnetic particles is added. Upon binding sclerostin, it orients sclerostin such that the C-terminal portion of sclerostin is distal to the capture particle's surface. A sequential separation and wash cycle is then performed to remove unbound sclerostin fragments and other non-specific serum/plasma proteins. Third incubation: Sandwich formation is achieved via addition of an ABEI-labeled polyclonal goat functionally anti-sclerostin C-terminal tail specific conjugate. Following a final separation and wash cycle, developer is added and emitted light photons [relative light units (RLU)] are measured and converted to pg/mL via a master standard curve. Total time to results is approximately 65 min. B. Specificity of the LIAISON sclerostin CLIA components overlaid upon a Clustal O(1.2.4) alignment of sclerostin and SOSTDC1 primary sequences. The capture monoclonal antibody is specific to a 16-mer located within the N-terminal tail (Gln_1 to Ser_{56}) of sclerostin. The tracer polyclonal antibody is conjugated to ABEI. Its functional C-terminal tail specificity is inferred from, and based upon, three observations: 1) The distal orientation of sclerostin's C-terminal portion consequent to N-terminal capture specificity of the PMP; 2) SOSTDC1's C-terminal portion (Gly_{117} to Ser_{206}) that aligns with the C-terminal thrombin peptide of sclerostin (Gly_{99} to Tyr_{190}) is twice as basic as its N-terminal portion (2.33fold [21.7%/9.2%] which is similar to sclerostin, whose C-terminal fragment is 2.23 fold [25%/11.2%] more basic than its N-terminal fragment; and 3) SOSTDC1, which has 47.1% direct homology to sclerostin's post-thrombin digest C-terminal Loop2-Loop3 fragment domain (Gly_{99} to Arg_{149}) and only 19.5% homology to the post-thrombin digest C-terminal tail domain (Phe_{150} to Tyr_{190}), does not inhibit the assay when added to the third incubation step. Collectively these observations make it more likely that the conjugate's specificity is predominantly towards the C-terminal tail. C. Primary attributes of sclerostin and SOSTDC1. Intact molecule: sclerostin (Gln_1 to Tyr_{190}) has 35.9% direct homology with SOSTDC1 (M_1 to Ser_{206}). Gly_{99} – Arg_{149} and Phe_{150} – Tyr_{190} comprise the Loop2–Loop3 and C-terminal portions of the C-terminal thrombin fragment, respectively. Percent basic (arginine/lysine) residue content of sclerostin secondary structural elements (i.e. Loops 1, 2 and 3) along with the N- and C-terminal thrombin fragments (and the aligned portions from SOSTDC1) demonstrate a similar relative basic aspect to the C-terminal portion of each molecule.

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