**Soluble CD44: A Biomarker for Cardiovascular Disease?**

Commentary to the article: Circulating soluble CD44 is higher among women than men and is not associated with cardiovascular risk factors or subclinical atherosclerosis. Metabolism 2005;54:139-41.

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**Introduction**

**Adhesion Molecule CD44 and Atherosclerosis**

For many years, the role of CD44 in neoplastic disease has undergone extensive scrutiny. Since some CD44-mediated cellular processes and signaling pathways have relevance to other pathophysiological conditions, several groups have undertaken study of CD44 in other malignant contexts, e.g. inflammatory and autoimmune disorders [1,2].

Expressed by both inflammatory and vascular cells, CD44 and its variants form a complex family of hyaluronate receptors whose molecules are produced by a single gene via splicing and post-translational modifications. Most CD44 ligands localize in the extracellular matrix (ECM). Hyaluronic acid (HA), the principal ligand for CD44, increases in the tunica media of macroscopically normal arterial walls in patients with type 2 diabetes. Clinical sequelae of atherosclerosis, e.g. myocardial infarction, angina pectoris, and stroke, occur more frequently in patients with type 2 diabetes, and their risk of developing cardiovascular disease (CVD) is 3-5 fold higher. However, the mechanisms for increased incidence of CVD are understood incompletely. Although established risk factors for CVD, such as hypertension and hyperlipidemia, occur more frequently in diabetic patients, the disease itself is an independent risk factor in both men and women. Indeed, recent work suggests that the metabolic and hormonal disorders of diabetes, i.e. elevated levels of glucose, insulin, and insulin-like growth hormone levels, stimulate CD44-mediated smooth muscle cell migration [3].

CD44 ligation induces several cell-cell and cell-matrix interactions that participate in the progression of atherogenesis, e.g. adhesion of activated lymphocytes to inflamed endothelium, homing, migration, cell activation, and apoptosis. No current evidence explains the mechanisms by which CD44 regulates apoptosis. Indeed, mouse studies have provided contradictory evidence that CD44 may either protect against [4,5] or promote [6] apoptosis. A very recent study shows that CD44 protects against death receptor-mediated (e.g. Fas/CD95) apoptosis in human cancer cell lines via mechanisms that may involve death receptor signaling and availability [7].

Recently, our group reported enhanced CD44 in human atherosclerotic disease and abdominal aortic aneurysm and particularly in macrophage (MΦ)-rich lesions containing features of unstable plaque. In such lesions, MΦ are the major source of CD44, and MΦ content correlates with CD44 protein levels [8]. Therefore, adhesion molecule CD44 may participate in inflammatory arterial diseases.

In 2001, Cuff et al. demonstrated that CD44 contributes directly to atherosclerosis in Cd44−/− mice. In that study, atherosclerotic lesion size decreased 50-70% in apoE-deficient Cd44−/−
mice compared with lesions from CD44 heterozygous and wild type mice [9]. Such data as well as many CD44-mediated cellular processes suggest CD44 as a potential therapeutic target in atherosclerosis.

**A Soluble Form of CD44**

In addition to its role as a transmembrane receptor on many cell types, the soluble form of CD44 (sCD44) localizes in both serum and lymph. sCD44 generates through proteolytic cleavage of cell surface CD44 [10] or by *de novo* synthesis due to alternative splicing [11].

Enzymes such as membrane-type 1 matrix metalloproteinase (MT1-MMP) [12], a chymotrypsin-like sheddase [13,14], serine-proteases, and MMPs [15] proteolytically cleave the extracellular domain of CD44, removing it from the cell surface. Such proteolytic enzymes occur frequently in atherosclerotic lesions, where they degrade ECM components. Additionally, such lesions commonly exhibit an imbalance of matrix-degrading enzymes and their inhibitors. The release of CD44 during inflammatory processes may indicate ongoing matrix remodeling due to enhanced proteolytic activity. Furthermore, both oncostatin M and transforming growth factor beta 1 (TGF-\(\beta\)1) may release CD44 from the cell surface. While sCD44 derived from oncostatin M shedding retains HA binding capacity, shedding induced by TGF-\(\beta\)1 produces sCD44, whose HA binding differs from that of cell surface CD44 [16]. Therefore, the mechanisms that govern CD44 release likely affect the binding of HA to the released receptor, thus influencing the cellular consequences of CD44 shedding.

Shedding of membrane-anchored CD44 likely alters cellular responses to the surrounding environment, since such cell surface modifications in addition to the potential influence of sCD44 on CD44-mediated HA binding to the cell surface affects CD44-mediated cellular processes. Although the exact affinity of sCD44 for HA remains unknown, sCD44 may inhibit CD44-HA-dependent cell-matrix interactions at the inflammatory site [17].

Since malignant diseases, immune activation, and inflammation associate with increased plasma levels of CD44, these processes may contribute to the release of CD44 from the cell surface. To date, the physiological inducers of CD44 release and the role of sCD44 remain incompletely defined.

Others have proposed soluble adhesions molecules as useful risk markers for cardiovascular disease [18,19]. However, the clinical relevance of soluble adhesion molecules and their potential as predictors of additional clinical risk remain undetermined. One promising candidate, soluble intercellular adhesion molecule-1 (ICAM-1) associates with carotid atherosclerosis progression [20] and also correlates with various risk factors for cardiovascular disease, e.g. smoking, hypertension, low HDL cholesterol, hypercholesterolemia, and hypertriglyceridemia [18]. Few data currently explain the relationship between sCD44, cardiovascular risk factors, and atherosclerosis. Accordingly, our study focused on examining these associations.

**Results**

**Study Subjects**

To test whether sCD44 is related to cardiovascular risk factors and atherosclerosis, we obtained subjects from two ongoing population-based studies at the Wallenberg Laboratory. The first
study compared circulating CD44 concentrations in non-diabetic 61-year old men (n = 142) with and without subclinical atherosclerosis [21]; the second examined sCD44 and intima-media thickness (IMT) in 64-year old women with normal glucose tolerance (n = 44) and diabetes mellitus (n = 16). Exclusion criteria for all groups included clinical atherosclerotic disease, smoking, chronic inflammatory disease, ongoing infection, C-reactive protein > 10 mg/L, and diabetes in the men. Because smoking induces sCD44, both studies excluded smokers [22].

Circulating sCD44 Levels in Women and Men

Levels of circulating sCD44 were higher in women than in men, a difference also observed in regression analyses with independent variables of either sex or age. Thus far, few studies have explored whether age affects sCD44 levels, and current publications suggest no relationship between sCD44 and age [23-25]. Compared with non-diabetic subjects among women, diabetes did not associate with higher sCD44. Furthermore, IMT did not associate with elevated sCD44 levels in women.

sCD44 levels were similar in men with and without subclinical carotid atherosclerosis. sCD44 and sICAM-1 correlated weakly in men (r = 0.28, P < 0.001) but not in women (r = 0.04, ns). We found no association between sCD44 levels and systolic blood pressure, LDL-cholesterol, HDL cholesterol, or triglycerides in men or women. Using National Cholesterol Education Program (NCEP) criteria of the National Heart, Lung, and Blood Institute, we further divided subjects into individuals with and without the metabolic syndrome (MS) [26]. sCD44 concentrations in men or women with MS differed from those without MS (367 ± 81 (mean ± SD) versus 347 ± 63 ng/mL in men with (n = 25) and without (n = 117) MS; 402 ± 116 versus 442 ± 141 ng/mL in women with (n = 16) and without (n = 44) MS).

Conclusion

Our study suggests that circulating sCD44 associates with neither cardiovascular risk factors nor subclinical atherosclerosis. To our knowledge, ours is the first report to address these issues. The higher sCD44 concentrations we observed in women do not corroborate a previous report based on a younger and less defined study population [25].

The venous blood used in our study was sampled far from the actual lesion. Since the total amount of sCD44 generated within the plaque may dilute within the blood circulation, such distance might influence the results. Blood sampling closer to the actual plaque may give a more accurate estimate of the actual amount of sCD44 generated under these pathological conditions.

In conclusion, a possible correlation between sCD44 and plaque burden remains undetermined. Clarifying the relation between sCD44, cell surface CD44, and atherosclerosis will require further investigation.

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References