The relationship between Thrombin and Osteopontin in Dupuytren’s Disease

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Objective Pathophysiology of Dupuytren’s disease (DD) remains unclear [1,2]. Thrombin is a multi-functional serine protease and a potent inducer of fibrogenic cytokines in various cells [3,4]. Osteopontin (OPN), one of ECM proteins, can also modulate a variety of cellular activities associated with various chronic inflammatory disease including myocardial fibrosis after ischemic heart disease, liver cirrhosis and lung fibrosis [5,6]. Additionally, the thrombin-cleaved form of OPN is well correlated with various inflammatory disease activities [7].

Purposes
1) To determine whether OPN, especially of thrombin-cleaved form (34E3), are expressed in DD
2) To investigate whether fibroblasts derived from DD are differentiated into myofibroblasts by the administration of thrombin

Materials and Methods

Patients: The study group consisted of 25 patients (4 women and 21 men) who underwent surgical resection of the palmer fascia for DD. The patients’ mean age was 69.1 years (range, 58 to 82 years). Furthermore, the palmer apponeurosis resected in five patients (mean age, 52.8 years) with carpal tunnel syndrome were used in as control.

Immunohistochemical analysis: Immunohistochemical studies were performed with antibody against αSMA, OPN(O-17) and OPN(34E3).

The determination of thrombin’s effect in vitro study: Cells isolated from nodules and cords were starved in serum-free medium overnight prior to treatment with thrombin, 1 U/ml. After 24 hours, expressions of αSMA and OPN were analyzed in total proteins collected from cells using western blotting.

Results

There was respective expression of OPN(O-17) and αSMA in 16 (67%) and 5 (20%) in cord. In addition, the expression of αSMA was significantly correlated with that of OPN(O-17) in nodules.

The proportion of myofibroblasts in cell cultures from nodule was 11.3%, compared with 4.6% in cord (P=0.011).

OPN(34E3) was immunolabeled on similar areas with OPN(O-17) in nodules, considered that the majority of OPN’s expression was thrombin-cleaved form in the nodules centered in the pathology.

After treatment of thrombin, expressions of αSMA and OPN were clearly upregulated in the cells from nodules as well as cords, although there were weak expression of these molecules without application of thrombin.

Conclusion This study showed myofibroblast expressed OPN and thrombin cleaved-form in the nodules as well as cords, with significant correlation of αSMA’s expression. Lenga described that OPN was required for the differentiation and activity of myofibroblasts based on the experiment using OPN-null fibroblasts [5]. Thus, OPN could involve in the pathologic progression by modulating the activity of myofibroblast in DD.

In addition, in vitro study showed the application of thrombin induced myofibroblast transformation from fibroblast of the nodules as well as the cords. Besides, expression of OPN was clearly upregulated in the cells from both nodules and cords. Thrombin in bleeding with the surgery may participate in the pathology of progression and recurrence by direct effect or indirect pathway via cleavage of OPN.

References