HUMAN CHONDROSARCOMA HCS2/8 CELLS EXPRESS TAURINE TRANSPORTER TRANSCRIPTS

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INTRODUCTION

Changes in cartilage hydration are known to occur during static compression, potentially causing a rise in the interstitial fluid osmolarity [1]. On the other hand, cartilage swelling is the first detectable macroscopic event in developing osteoarthritis [2]. These changes in extracellular matrix hydration can be assumed to alter chondrocytes' volume, too. It is known that regulation of constant cell volume is important for chondrocytes to maintain their optimal metabolism and biosynthesis of extracellular matrix molecules. Chondrocytes respond to anisotonic solutions by controlling the rate of water transport across the plasma membrane. This transport is fast, so that increase or decrease of extracellular osmolarity correspondingly decreases or increases cell volume within seconds [3]. To limit these changes chondrocytes have specific membrane transporters which release or accumulate solutes in response to cell swelling or shrinkage, respectively [4,5]. Taurine transport is one mechanism to regulate the cell volume [6]. We have used differential display RT-PCR technique to screen for alterations in mRNA profiles in human chondrosarcoma cells exposed to cellular stretching. One of the clones that we obtained was highly homologous with previously reported canine taurine transporter. In this study, we further characterize its expression in human chondrosarcoma cells in relation to a number of other human tissues and cells.

METHODS

Human chondrosarcoma HCS2/8 cells [7] were cultured under standard cell culture conditions on silicone dishes. The cells were exposed to 1% cellular stretching at a frequency of 1 Hz. Total RNA was extracted from the control and stretched cells, and differential display RT-PCR was performed using 3'-anchored and arbitrary primers. Differentially up-regulated DNA product was cut off from the gel, re-amplified by PCR, and analyzed using single-strand conformational polymorphism (SSCP) technique. The most strongly induced DNA fragment in SSCP was cloned and sequenced. The tissue-specific expression of the corresponding transcript was investigated using multiple tissue and cancer Northern blot membranes provided by Clontech.

RESULTS

Differential display RT-PCR revealed a differentially expressed DNA product that was maximally induced after 3 h of cellular stretching. SSCP analysis showed that the band obtained in the first stage by differential display RT-PCR contained at least three different DNA fragments (Fig. 1A). The most strongly expressed band of these (Fig. 1A, arrow) was cloned and used as a probe to confirm that it hybridizes with specific transcripts on Northern blot. Indeed, one band was observed corresponding to 7.0 kb product in human chondrosarcoma cells (Fig. 1B).

DNA sequence of the 155 bp stretch clone (SC) had a complete similarity with canine taurine transporter, and weaker homology with the cDNA of expected human taurine transporter expressed in nervous tissue (Fig. 2). The size estimated by Northern analysis matched with the size of the canine taurine transporter (6.9 kb).

Analysis of tissue-specific expression of taurine transporter revealed a considerable expression in leukocytes, lung, placenta, kidney, spleen, and heart. In muscle and brain the expression was moderate, while intestine, liver and thymus showed only a low level of expression. All of the cancer cells investigated here expressed the taurine transporter.

DISCUSSION

In this report, we show that human chondrosarcoma cell line expresses a 7.0 kb transcript of taurine transporter. A similar size transcripts were observed in a number of other human tissues, too. Cyclic stretching induced taurine transporter in differential display RT-PCR, however, the result must be further confirmed by other methods.

REFERENCES

1. Mow VC, Ratcliffe A, Poole AR. Biomaterials 13, 67-97, 1992

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