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EXPRESSION OF MUSCARINIC ACETYLCHOLINE RECEPTOR mRNA IN PRIMARY CULTURE OF HUMAN PROSTATE STROMAL CELLS AND EPITHELIAL CELLS .

Aims of Study: Muscarinic acetylcholine receptors(mAchRs) belong to the family of seven-transmembrane domain receptors that transduce extracellular neurotransmitter signals activating G proteins within the cell. Five mAchR subtypes have been identified and most tissues that have muscarinic activity express multiple subtypes. The aim of this study was to investigate the expression of mAchR subtypes mRNA in primary culture of human prostate stromal cells and epithelial cells using the reverse transcription / polymerase chain reaction (RT-PCR), RNA blotting and in situ hybridization (ISH).

Methods: Human normal prostates were obtained from patients undergoing radical prostatectomy due to prostate cancer. We obtained a primary culture of prostate stromal cells and epithelial cells by explant method from 3 normal prostates. Total RNA were extracted from each cell cultures using Acid Guanidinium method for cDNA synthesis. First-strand cDNA was then used for PCR with primers designed to amplify the fragments of each mAchR subtypes (m1-m5) cDNA sequence.

Results: In stromal cell cultures, the m2,m3 and m4 subtype expected bands were detected. Especially, m2 transcripts was strongly detected. Each PCR products were subcloned into the pGEM-T plasmid vector, sequenced and randam primer labeled using ^{32}P. Labeled probe was hybridized. Digoxigenin labeled cRNA probes were synthesized by in vitro transcription. RNA blotting using a m2 muscarinic receptor cDNA probe revealed a 4.5kb single transcript. However, m3 and m4 probes did not hybridize. By ISH, m2 receptor mRNA signals were detected in several smooth muscle cells. The staining were predominantly localized to the perinuclear cytoplasm. m3 and m4 probes did not hybridize.In epithelial cell cultures, the m1,m2 and m5 transcripts were observedby RT-PCR.However,any positive signals were not detected following RNA blotting and ISH.

Conclusions: These results suggested that m2 receptor subtype plays a role in smooth muscle activity in the human prostate.