[S2-2] [4/18/2002(Thur) 14:30-15:00/Hall B]

Signal Transduction of a Metastasis-Enhancing Mitogen, Autotaxin -

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Cell motility is a fundamental process required during normal embryonic development, inflammatory responses, wound healing, and tumor metastasis [1]. Autotaxin (ATX) is a 125-kDa glycoprotein initially isolated from the human melanoma cells [2]. This autocrine motility factor stimulates pertussis-sensitive chemotaxis in human melanoma cells at picomolar to nanomolar concentrations. DNA sequence analysis determined that ATX was homologous to a family of exo/ecto NPPs that includes the B cell activation marker, PC-1, and the neural differentiation antigen, B10 [3]. Among multiple enzymatic activities of ATX, the phosphodiesterase (PDE) activity appears to be essential for motility stimulation, since a single point mutation at threonine-210 abolishes the PDE- and tumor cell motility-stimulating activities of ATX [4].

A recent study has revealed that combination of ATX expression with ras transformation produced cells with greatly amplified tumorigenesis and metastatic potential compared to ras-transformed controls. Thus, ATX appears to augment cellular characteristics necessary for tumor aggressiveness [5]. Furthermore, ATX stimulates HUVECs grown on Matrigel to form tubules, much like vascular endothelial growth factor [6], suggesting that ATX could contribute to the metastatic cascade through multiple mechanisms.

Although ATX has been demonstrated to act as a autocrine motility factor in tumor cells, little is known about the signaling mechanism by which ATX stimulates cell motility. Therefore, the clarification of the possible involvement of specific signaling molecule in ATX stimulation of motility would be important for understanding the molecular mechanism of tumor invasion and metastasis. In the present study, we investigated the possible involvement role of phosphoinositide 3-kinase (PI3K) in ATX-mediated tumor cell motility stimulation and found that G protein-coupled PI3Kγ plays a pivotal role in the stimulation of motility by ATX in human melanoma cells [7]. Pretreatment of human melanoma cells with wortmannin or LY294002 inhibited ATX-induced motility (Figure 1). PCR amplification and immunoblot analysis showed that human melanoma cells appear to express both mRNA and protein for p110γ (Figure 2). ATX increased the PI3K activity in p110γ, but not p85, immunoprecipitates (Figure 3A). This effect was abrogated by PI3K inhibitors or inhibited by pertussis toxin (Figure

3B). Furthermore, stimulation of tumor cell motility by ATX was inhibited by catalytically inactive form of PI3Kγ (Figure 4), strongly indicating the crucial role of PI3Kγ for ATX-mediated motility in human melanoma cells.

Reference

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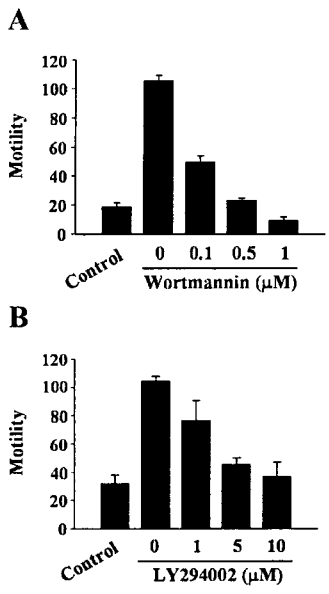

Fig. 1. Effects of PI3K inhibitors on ATX-mediated tumor cell motility.

Fig. 2A

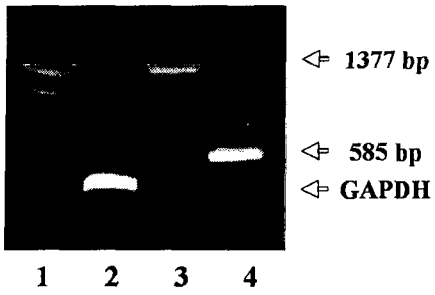

Fig. 2B

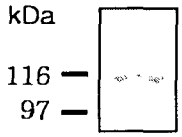

Fig. 2. Presence of p110g in human melanoma cells. A. RT-PCR. Lane 1: Molecular weight markers (ΦX174 DNA-*Hae*III digest); lane 2: GAPDH; lane 3: 1377 bp DNA fragment of p110γ; lane 4: 585 bp DNA fragment of p110γ. B: immunoblott analysis.

Fig. 3A

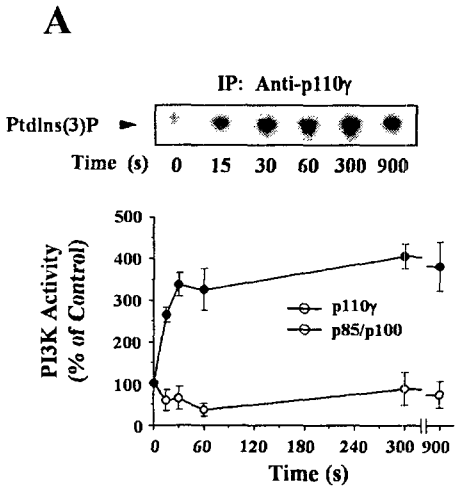

Fig. 3B

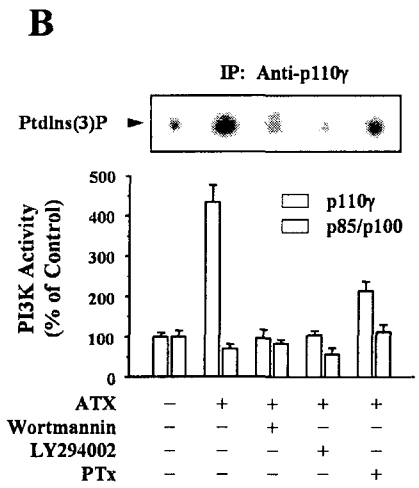

Fig. 3. Activation of p110γ by ATX in A2058 cells. A: Stimulation of the activity of p110γ by ATX in various times. B: Effects of wortmannin, LY294002, and PTx on the stimulation of p110γ activity by ATX. The upper panel depicts the result of a representative TLC plate and the lower panel shows the average densitometric values.

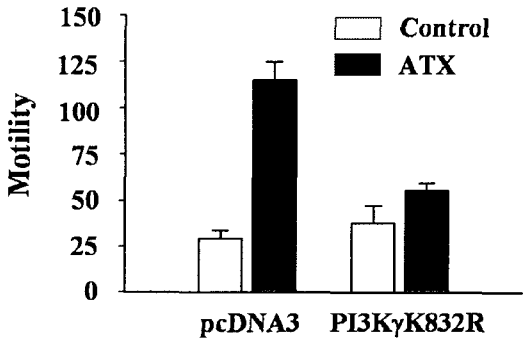

Fig. 4. Involvement of PI3Kγ in ATX-induced tumor cell motility.