C-MET ONCOGENE IN HUMAN CHORDOMA CELLS

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Introduction: Chordomas are primary tumors that arise from notochordal remnants along the axial skeleton. They are highly resistant to radio- and chemotherapies, and eventually cause death by direct growth or metastasis. The Met tyrosine kinase receptor and its ligand, hepatocyte growth factor (HGF), play important roles in both normal development and in tumor growth and metastasis. Chordomas have been found to strongly express c-Met [1]. Here we present a new chordoma cell line established from a primary sacral tumor, and novel data on c-Met.

Materials and Methods: A human chordoma cell line was established from a primary sacral tumor of a 78-year-old female. Primary cells were isolated using a modified enzymatic digestion protocol [2,3]. Cells were cultured in DMEM/F-12 with 10% FBS and probed for chordoma markers. Cells were lysed and total proteins were resolved by SDS-PAGE [4], followed by Western blotting. For tyrosine protein phosphorylation blots, cells were stimulated with HGF (30 ng/ml) in serum-free media for 15 min at 37°C. For cell migration, a transmigration chamber was utilized and cells were stimulated with HGF (30 ng/ml). Migration was blocked with an anti-c-Met antibody. FITC-conjugated anti-rabbit and Texas Red anti-mouse were used for immunostaining of c-Met and HGF.

Results: Western blotting with anti c-Met showed the presence of two bands, 85 and 45 kDa, corresponding to α and β subunits of c-Met respectively (Fig 1A). Immunoblotting of tyrosine phosphorylated proteins revealed changes in phosphorylation levels in HGF stimulated cells (Fig 1B). HGF increased expression of proteins, MW 45, 57 and 62 kDa, and induced tyrosine-phosphorylation of new proteins, MW 82 and 90 kDa. Two bands corresponding to the HGF large and small subunits of 62 and 34 kDa respectively were obtained by WB and anti-HGF in stimulated and non-stimulated cells. External HGF stimulation did not affect the amount of cellular HGF (Fig. 1C). In unstimulated cells, immunostaining detected nuclear and membrane/cytoplasmic localization of c-Met and membrane/cytoplasmic localization of cellular HGF with their colocalization in cytoplasm. The HGF-stimulated cells showed nuclear and perinuclear presence of c-Met with its colocalization with HGF within perinuclear region (Fig 2). Active cell migration was induced by HGF and abolished by the anti-c-Met antibody (Fig 3).

Discussion: Our results show that the HGF receptor, c-Met, is constitutively expressed by human chordoma cells. c-Met is functionally active and drives migration towards a HGF gradient in vitro. This suggests that c-Met may play an important role in chordoma cell migration and metastasis. Moreover, native HGF is steadily co-expressed by chordoma cells, and its expression level is not affected by exogenous HGF. Our results suggest a mechanism for chordoma metastasis: in vivo, one cell population may produce and release high amounts of HGF, whereas another population in the same tumor may overexpress the c-Met receptor and migrate toward HGF chemotactic gradients in remote body sites, forming metastatic lesions.

Aberrant protein tyrosine phosphorylation is a hallmark of many types of cancer, and plays a key role in regulating many different processes such as growth, cell cycle control, differentiation, cell movement, and gene transcription. In our study, the amount and number of tyrosine phosphorylated proteins were significantly increased upon HGF stimulation, and thus such altered intracellular signaling may be directly involved in chordoma malignancy. Translocation of c-Met and HGF to the nucleus may be one factor that causes altered intracellular signaling in chordoma.


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Figure 1. WB of c-Met subunits (A); WB of tyrosine phosphorylated proteins (B) and HGF (C) in the presence or absence of HGF stimulation

Figure 2. Colocalization of c-Met and HGF with and without HGF stimulation

Figure 3. Chordoma cell transmigration toward HGF. Anti-c-Met blocks cell migration