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Midkine, A potential Tumor Biomarker is Enhanced in Huh7 And HepG2 Human Hepatoma Cells

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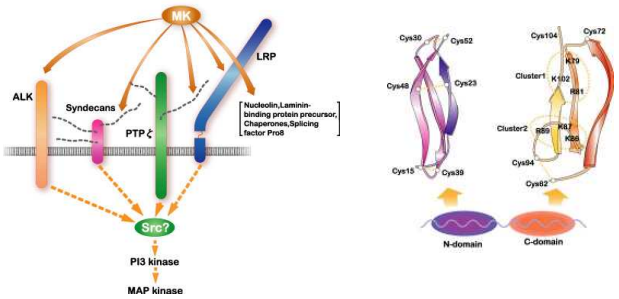
ABSTRACT

Midkine (MK) is currently discovered as a new member of a unique heparin-binding neurotrophic factor or cytokine family which is composed of MK and PTN (Pleiotrophin). MK plays important roles in development, neuronal survival and differentiation, tissue remodeling and carcinogenesis. The augmented expression of midkine has been revealed in many advanced tumors at very high frequency in non-tissue specific manner. The serum midkine level is increased in many cancer patients, making the detection level of MK a potential tumor marker in clinical diagnosis.

We have developed immunoassays to detect midkine based on a panel of high affinity and specificity antibodies against human midkine. The Huh7 and HepG2 hepatoma cells were grown in high-glucose DMEM with essential supplements. The culture medium was collected at 4th passage with 80% confluence for midkine ELISA detection. The protein extracts were prepared from 10^7 cells/ml by adding lysis buffer, half of the samples were used for midkine ELISA detection, and the other half were fractionated by sodium dodecyl sulfate-polyacrylamide gel eletrophoresis (10% SDS-PAGE). We found that the antibodies can recognize ~14 kDa protein from the hepatoma cell lysates, these immunoreactive bands can be blocked by recombinant protein midkine. Further more, MK levels are detectable in the hepatoma cell culture medium (12.6ng/ml, n=20, CV=9.8%) as well as the cell lysate preparations (15.1 ng/10^6 cells, n=10, CV=14%) compared to the non-detectable MK levels from the negative controls.

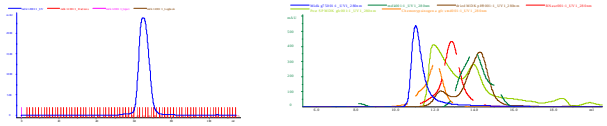
In conclusion, the fast and sensitive ELISA method for midkine detection can be a potential tool for clinical diagnosis. The attempt of targeting midkine might be a plausible approach to illustrate the midkine in anti-apoptotic, angiogenic and other carcinogenesis-related activities.

The signal receptor complex of midkine (MK) and downstream signaling system



MATERIALS AND METHODS

1: Midkine recombinant protein construction and purification



2: Antibody development and characterization

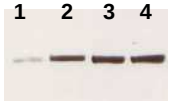
Sample O.D	Prebleed Mix of rabbit sera		Test Bleed Mix of Antisera		AP Antibody F1		AP Antibody F2		Running Through Waste		NA	
Dilution	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1.1K	0.236	0.243	1.767	1.681	1.834	1.766	0.751	0.627	1.172	1.202	0.097	0.091
1.5K	0.143	0.123	1.103	0.995	1.077	1.028	0.393	0.357	0.198	0.191	0.077	0.081
1.25k	0.099	0.113	0.973	0.912	0.914	0.922	0.233	0.238	0.154	0.132	0.099	0.115
1.125K	0.125	0.124	0.407	0.408	0.834	0.896	0.172	0.165	0.133	0.128	0.101	0.094
1.625K	0.116	0.139	0.164	0.181	0.435	0.373	0.22	0.224	0.103	0.094	0.109	0.092
1.3125K	0.103	0.091	0.091	0.103	0.174	0.158	0.108	0.096	0.089	0.089	0.108	0.091
1.1562K	0.115	0.094	0.089	0.098	0.108	0.113	0.097	0.102	0.102	0.107	0.091	0.094
1.0781K	0.104	0.085	0.085	0.078	0.078	0.087	0.084	0.077	0.09	0.083	0.085	0.078

1-WB on purified MK recombinant protein
2-Blocking by MK recombinant protein

3:Cell culture of Hep G2 and Huh7, cell lysate preparation

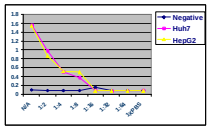
Hep G2 and Huh 7 cells were grown in DMEM with 4 mM L-glutamine, 1.0 mM Sodium pyruvate, and 10%FBS at 5%CO2, 37oC. When the cells became 80% confluence, lifted cells by Trpsin-EDTA, and seeded into new vessels. All the samples and cell lysate preparation were performed at p4.

4: Western blot



1-T.C. Super; 2-Huh 7 cell lysate
3: -HepG cell lysate, 4-Positive control

5: Midkine protein level determination by ELISA



CONCLUSIONS

Midkine (MK) is discovered as a multifunctional growth factor and plays important roles in carcinogenesis. The intercellular cytoprotective signals mediated by midkine, may develop resistance to drug cytotoxic agents, which limiting clinical use to treat human cancers. Although there is no evidence to clarify the relationship between elevated level of MK and cancers. In our study, we developed a high affinity and specific antibody against Midkine, which is very useful for immunoassay. The fast and sensitive ELISA method for midkine detection can be a potential tool for clinical diagnosis. Further more, blocking the activation/function of MK, may lead a new area for drug discovery and contribute to multi-cancer treatments.

REFERENCES

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2: Mirkin,B.L., et al (2005) Identification of midkine as a mediator for intercellular transfer of drug resistance. Oncogene 24 (31), 4965-4974

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