

HGF/SF-Induced Ca^{+2} Intake to Breast Tumor Cells – a Manganese Enhanced MRI Study

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INTRODUCTION

Targeted biological therapies are evolving rapidly, leading to an increasing need for the development of imaging modalities to evaluate patient susceptibility and response to personalized therapy. Here we report a novel functional molecular imaging modality that may be used for monitoring response to anti Met tyrosine kinase targeted therapy. Aberrant signaling through Met tyrosine kinase receptor and its ligand hepatocyte growth factor/scatter factor (HGF/SF) has been described in a variety of human cancers. Phase II and III clinical trials using various anti Met therapy showed encouraging results in several solid tumors. HGF/SF-induced Met activation leads to diverse metabolic alteration in the tumor including increased oxygen and glucose consumption, glycolysis, and cytosolic NADH/NAD ratio (1). Using BOLD contrast MRI and contrast media ultrasound we demonstrated that HGF/SF treatment dramatically increased blood volume in murine mammary tumors expressing Met (2,3).

Manganese is a paramagnetic contrast agent that enters the cells due to the permeability of voltage-gated calcium channels (4). Manganese enhanced MRI (MEMRI) enables monitoring functional cellular calcium intake via *in-vivo* mapping of Mn^{+2} ions. It was previously shown that calcium intake to the cells plays an important role in HGF/SF induced cell migration *in vitro* (5). Here we use MEMRI to investigate the alteration of calcium intake upon activation of HGF/SF Met signaling in mammary tumors as a possible novel Met functional molecular imaging modality.

METHODS

Cell culture and tumor induction: DA3, a poorly differentiated Met dependent murine mammary adenocarcinoma cells, were injected into the mammary gland of BALB/C mice.

Mn^{+2} , HGF/SF and Verapamil administration: MnCl_2 (50 mM/kg body weight) was administered *i.p.* 10-12 hours before the MRI scans. HGF/SF (100ng/gr) was injected *iv* through a catheter to the tail vein during MRI scans. Verapamil (3.5-5mg/kg) was *ip* injected 15 min before HGF/SF.

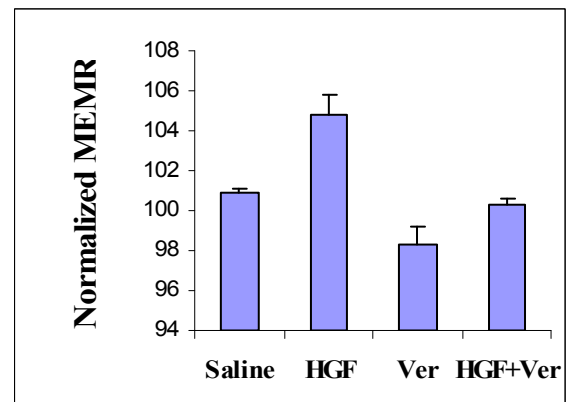
MRI: measurements were performed using a 3T whole body MRI system (3T GE, HDx), mice volume coil (4.5 cm in diameter), and FSE T_1w sequence with TR/TE=360/4 msec, SW 2 mm, FOV 5 cm, 256X160 matrix, in plane resolution 195 μm .

MEMRI signal intensities were measured in ROIs including the whole tumor before and after *i.v.* injection of HGF/SF or saline, with or without the Ca^{+2} blocker Verapamil.

RESULTS AND DISCUSSION

To better understand the role of HGF/SF in tumor Ca^{+2} intake, Mn^{+2} -treated mice were intravenously injected with HGF/SF; saline injection served as control. Changes in Mn^{+2} enhanced signal intensity were evaluated from T_1 weighted images acquired in 1-min intervals for 40 min. Analysis of signal intensity alteration was performed by comparing base line intensity (MEMRI-bl -average of first 7-10 images), to maximum signal change (MEMRI-max -average of 7 images taken 20 min. after HGF/SF injection), where Normalized MEMRI = the ratio (MEMRI-max / MEMRI-bl)*100. Fig 1 demonstrates that HGF/SF treatment induces 4% normalized MEMRI enhancement (n=16, $p<0.001$), i.e., increased Mn^{+2} fluxes, while in the saline-injected mice no enhancement was observed. To verify that the effect is due to Mn^{+2} intake into the cells and not due to the increase in blood volume, mice were treated with different doses of Verapamil (Ver), a Ca^{+2} blocker, and 15 min later treated with HGF/SF. Verapamil treatment alone caused a 2% decrease in normalized signal intensity. The analysis of HGF/SF effect following verapamil injection demonstrated blocking of 50% of the HGF/SF enhancement (n=12, $p=0.0007$) under our experimental conditions.

Our hypothesis is that HGF/SF activates Met in tumor cells, resulting in enhanced Mn^{+2} intake into the cells through Ca^{+2} channels, which is blocked by verapamil. These results further demonstrate the dramatic physiological alteration in the tumor upon activation by HGF/SF. Moreover, that HGF/SF MEMRI may serve as a Met functional molecular imaging modality for monitoring anti Met treatment.



CONCLUSIONS

1. MEMRI is a sensitive modality for studying physiological effect on Ca^{+2} intake in tumors.
2. HGF/SF induces enhanced intake of Mn^{+2} to Met-dependent mammary tumor cells that is inhibited by the Ca^{+2} blocker Verapamil.
3. HGF/SF-induced MEMRI signal may serve as a novel Met functional molecular imaging modality for monitoring anti Met therapy.

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