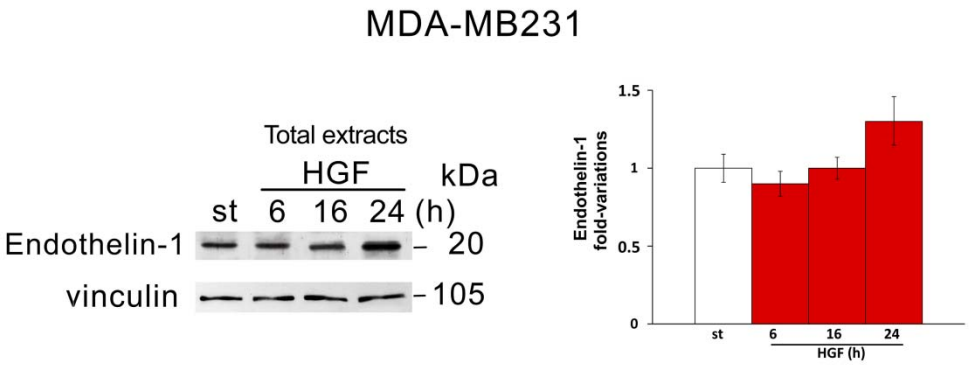
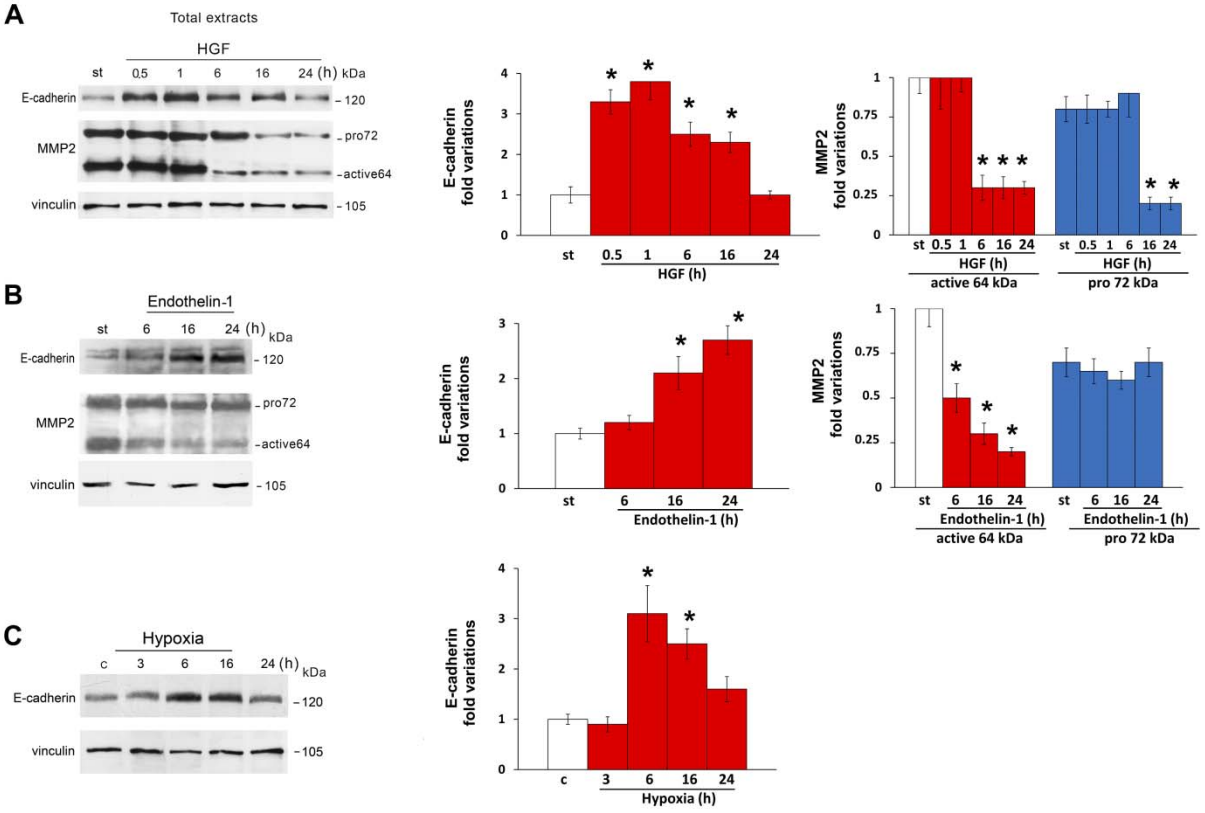
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Endothelin-1 expression under HGF in MDA-MB231 cells. One of the target genes of Ets-1 is Endothelin-1 [1]. We evaluated whether HGF influenced the pattern of Endothelin-1 in parental MDA-MB231 breast carcinoma cells. A representative Western blot is reported. Vinculin was used for the normalization of the data. As shown in the histogram (means±S.E. of three independent experiments), we observed that Endothelin-1 steady state protein level was unaffected by HGF treatment.



Supplementary Figure 2. Pattern of E-cadherin and MMP-2 under HGF, Endothelin-1 and hypoxia. We evaluated the effects of various microenvironmental stimuli on molecules critical for bone metastasis phenotype, and Western blot experiments were performed [1-3]. 1833 cells under starvation were exposed to 100 ng/ml of HGF or to 50 ng/ml of Endothelin-1. HGF enhanced E-cadherin showing a peak at 0.5-1 h, while the precursor (72 kDa) and the active forms of MMP-2 progressively decreased. Similarly, Endothelin-1 oppositely affected E-cadherin and MMP-2-active form expression. Also, the physical stimulus hypoxia (1% O_2) augmented E-cadherin expression between 6 and 16 h. Altogether, the data indicated that the microenvironment might influence the phenotype of 1833 metastatic cells through biological and physical stimuli, and it seemed to enhance the epithelial characteristics reducing the invasive/mesenchymal behavior. However, in the present paper we observed a complex interaction of the stimuli, and HGF down-regulated Endothelin-1 transactivation and protein expression by augmenting HIF-1α.

References

1. Bendinelli. P.; Maroni, P.; Matteucci, E.; Luzzati, A.; Perrucchini, G.; Desiderio, M.A. Microenvironmental stimuli affect Endothelin-1 signaling responsible for invasiveness and osteomimicry of bone metastasis from breast cancer. *Biochim. Biophys. Acta* **2014**, *1843*, 815-826.
2. Bendinelli. P.; Maroni, P.; Matteucci, E.; Desiderio, M.A. HGF and TGFβ1 differently influenced Wwox regulatory function on Twist program for mesenchymal-epithelial transition in bone metastatic versus parental breast carcinoma cells. *Mol. Cancer* **2015,** *14*, 112.
3. Maroni, P.; Matteucci, E.; Drago, L.; Banfi, G.; Bendinelli, P.; Desiderio, M.A. Hypoxia induced E-cadherin involving regulators of Hippo pathway due to HIF-1α stabilization/nuclear translocation in bone metastasis from breast carcinoma. *Exp. Cell Res.* **2015**, *330*, 287-299.