http://dx.doi.org/10.7124/bc.000AF7

Alteration in the ratio of S6K1 isoform expression modifies the response of cancer cells to the influences of the tumor microenvironment

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Aim. This work aimed to clarify the impact of mTOR/S6K1 cell signaling, with an emphasis on S6K1 isoforms, in the response of tumor cells to fibroblast influence *in vitro*. **Methods.** Cell Culture and Co-Culture: Tumor cells were cultured both alone and co-cultured with human dermal fibroblasts. Immunofluorescence Analysis: Used to detect specific proteins and observe cellular changes. Western Blot Analysis: Employed to analyze protein expression and signaling pathways. Scratch Test and Spheroid Spreading Assay: Assessed cell locomotion and the dynamics of spheroid spreading. **Results.** *mTOR/S6K1 Signaling:* Fibroblasts positively affected the activity of the mTOR/S6K1 signaling network, including p85S6K1, p70S6K1, p60S6K1, and mTOR in MCF-7 breast carcinoma cells. *Cancer Cell Locomotion:* Fibroblasts promoted cancer cell movement in a paracrine manner, as shown in scratch tests and spheroid spreading assays. Direct contact with the fibroblast monolayer significantly reduced the velocity of spheroid spreading. *Isoform-Specific Effects:* Using MCF-7 cell lines with edited expression of S6K1 isoforms, it was found that selective expression of p60S6K1 altered the morphological properties of cancer cells in 2D and 3D cultures. These cells also showed higher levels of focal adhesion kinase (FAK) phosphorylation and increased content of Zo-1, CD29, and CD44. *Resistance to External Factors:* The cells selectively expressing p60S6K1 were resistant to fibroblast-produced factors and rapamycin. **Conclusion.** The data highlight the significance of the ratio of S6K1 isoform expression in cancer cells for their response to microenvironment factors.

K e y w o r d s: S6K1 isoforms, tumor microenvironment, cell locomotion.