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Gab Proteins and RON-mediated Signaling
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ABSTRACT

RON, (recepteur d' origine nantais), is a receptor tyrosine kinase that can cause the increased growth, invasion and metastasis of tumors when over expressed in breast epithelial cells. When phosphorylated by the ligand Macrophage-Stimulating Protein, (MSP), RON signals the Gab adaptor proteins, Gab1 and possibly Gab2. These proteins then heighten cellular signaling, which may contribute to cellular transformation. Gab1 is necessary to carry on cellular processes and when compared to normally functioning breast epithelial cells it is not over expressed in breast cancer carcinomas. However, studies with v-Sea, a RON homolog found in chickens, reveal that Gab 2 influences the transformation of fibroblasts. The current study uses western blotting and immunofluorescence techniques with MCF10A and cos1 cell lines to explore how the Gab adaptor proteins contribute to RON-mediated signaling. I hypothesizes that Gab2 is the dominating protein, but Gab1 is also necessary to induce the progression of cancer cells. Insight into how Gab1 and/or Gab 2, work together to cause the transformation, proliferation, and metastasis of normal cells will provide a potential target in the treatment of breast cancer carcinomas.

INTRODUCTION

Breast cancer is one of the most popular forms of cancer among women in the United States. It is the second leading cause of all cancer deaths among women nationally (American Cancer Society). Like most manifestations of the disease, breast carcinomas are marked by increased proliferation of mutant cells. In addition to an increased ability to adhere to other cancerous cells and organs in the body, these genetically altered cells show a decrease in their ability to undergo cellular senescence and programmed cell death or apoptosis all of which contribute to the invasion and metastasis of breast epithelial tissue by cancer cells.

Cellular signaling, influenced by tyrosine phosphorylation of receptor tyrosine kinases (RTKs) is a major factor that influences cell cycle functions including cell migration, metabolism and survival, differentiation, and proliferation (Schlessinger). One of these RTKs, RON (recepteur d'origine nantais) has been implicated in the progression and metastasis of tumors in breast carcinomas. When RON binds to its ligand Macrophage Stimulating Protein (MSP) it stimulates the downstream signaling cascades MAPK and P13K that then provide docking sites for molecules such as the Gab adaptor protein Gab1 and, possibly, Gab2. Studies in v-Sea, a RON homolog found in chickens, reveal that Gab2 influences the transformation of fibroblasts and is required for the BCR/ABL fusion protein found in human leukemias (Hayman and Maroun). In contrast, although Gab1 is necessary for cell survival and it is known to be stimulated by RON activation, Gab1 is not over expressed in breast carcinomas when compared to normal breast epithelial cells and the role the Gab adaptor proteins play in RON-mediated signaling remains a mystery.

To gain insight into how Ron uses Gab1 and Gab2 in downstream signaling we prepared cell lysates in 3T3, MCF10A, and Parental MCF10A cell lines + or - MSP and RON as well as performed immunofluorescent techniques on the cos1 cell line inserted with expression vectors for HA-Gab2, and Gab1/RON. Analysis of the results will lead to potential targets for treatment in breast carcinomas by providing information on how Gab1 and/or Gab2 work in conjunction with RON to cause the increased transformation, growth, and metastasis of breast carcinomas.

RESULTS

Tyrosine kinase activity. 4G10m, an anti-phosphotyrosine was used to detect all tyrosine-phosphorylated proteins in the cell lines used (Fig. 4). We probed for phosphorylated AKT using pAkt primary and anti-rabbit secondary. We detected phospho-AKT in the cell lines NIH3T3 and MCF10A where RON was over expressed and contained the ligand MSP (Fig. 1, Lanes 1,3,5,6). Also, phosphorylated MSP was detected in cells over expressing RON activated by MSP (Fig.2, Lanes 5 and 6). We probed for MAPK and RON as a control (Figures 5 and 6). As expected every lane became phosphorylated.

Signaling of Gab1. We transfected Cos1 cells with Flag-labeled Gab1 and prepared them for immunofluorescence. Gab1 was detected mostly along the outer regions of the cell (Fig. 3)

DISCUSSION

We have used 4g10m to demonstrate that tyrosine phosphorylation occurs in the cell lines used (Fig. 4). When RON was over expressed in cells containing MSP activation occurred (Fig. 1, Lanes 1,3,5,6). MCF10A, which contained only endogenous RON showed no activation (Fig. 1, Lanes 2, 4). Although RON was phosphorylated in

NIH3T3/RON-MSP cells when probed for pMAPK, more activation occurred in those cells that contained MSP (Fig. 2, Lanes 5,6). Therefore, we know that binding of RON to the ligand MSP is necessary to begin phosphorylation events in the cell. We also, with the use of pAkt and pMAPK demonstrated that RON is essential in the activation of P13K and MAPK signaling cascades. Figure 3 shows that Gab1 is mostly localized around the circumference of Cos1 cells. This indicates that Gab1 and RON somehow work together to signal downstream proteins and may contribute to the increased growth and metastasis of breast carcinomas.

CONCLUSION

MSP is essential in RON activation. Without RON phosphorylation the P13K and MAPK signaling cascades do not begin. Phosphorylation events are unable to take place in the cell and expression of genes controlling cell functions such as survival and growth (P13K & MAPK) are inhibited.

