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PRINCIPAL INVESTIGATOR: Ulrico Rodeck, M.D.

CONTRACTING ORGANIZATION: Thomas Jefferson University
Philadelphia, Pennsylvania 19107

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Ulrico Rodeck, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Thomas Jefferson University
Philadelphia, Pennsylvania 19107

E-Mail: Ulrich.rodeck@jefferson.edu

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Background: Signaling through the epidermal growth factor (EGFR) has been implicated in both effective wound healing and epithelial neoplasia. We have identified a novel function of the EGFR in support of epithelial cell survival, particularly in conditions of anchorage-independence. Furthermore, we have implicated MEK/MAPK signaling in this process.

Objective/hypothesis: Define molecular mechanisms and pathways by which EGFR activation supports epithelial cell survival. Two specific aims focus on (1) posttranslational modification of relevant Bcl-2 family members by EGFR activation through MAPK-dependent mechanisms and, (2) STAT3 activation by deregulated EGFR signaling as observed in epithelial cancer.

Progress: During the funding period from March, 2004 to March, 2005 we have completed a study on posttranslational modification of BIM, a pro-apoptotic family member of the Bcl-2 family through ERK/MAPK. This work is relevant to SA1 of the original application and has been submitted and is currently being revised to be published in Oncogene. In addition, we have further characterized signaling through the MAPKinases JNK and p38 as it relates to EGFR activation in the anchorage-independent state.

14. SUBJECT TERMS
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INTRODUCTION

The epidermal growth factor receptor (EGFR) is a type-1 receptor tyrosine kinase. Activation of the EGFR has been implicated in many aspects of cell biology pertaining to wound healing and malignant transformation of epithelial cells. Previous work from our laboratory underscored a novel function of the EGFR in support of cell survival of epidermal keratinocytes. Specifically, EGFR activation and signaling was observed to protect keratinocytes against induction of apoptosis through extracellular stressors (1, 2). The major thrust of the research funded through **DAMD17-02-1-0216** is to further characterize the protective role of EGFR activation in situations in which keratinocytes encounter suboptimal extracellular matrix interaction, i.e. during wound healing and metastatic spread of malignant cells. We originally proposed to pursue two specific aspects of EGFR activation as they relate to cell survival. Specific Aim 1 focuses on posttranslational modifications of regulators of cell survival of the Bcl-2 family of molecules through EGFR activation and suspension culture. Specific Aim 2 deals with the question whether inappropriate signaling through the EGFR as observed in cancer cells leads to aberrant STAT3 activation, which in turn enhances cell survival.

BODY

This progress report will focus on accomplishments relating to Specific Aims 1 and 2 of the original proposal as we have made significant progress in both areas. A manuscript detailing results relating to Specific Aim 2 has been published in Cancer Research in 2004. A second paper specifically dealing with Specific Aim 1 has been submitted for publication.

Specific Aim 1

We have evaluated posttranslational modification of the proapoptotic Bcl-2 family member BIM during suspension culture and in the presence and absence of EGFR activation in HaCaT keratinocytes. These studies were described in detail in the previous progress report. In addition to the results described earlier, we have identified MAPK activity to be critical for posttranslational modification and proteasomal degradation of BIM. We have further demonstrated that PKC-δ and MAPK cooperate to phosphorylate BIM and keep its expression level low. This work has been submitted for publication: *'EGFR-dependent downregulation of*

BIM in epithelial cells requires MAPK and PKC- activities' by Marlene R. Quadros, Sharon Connelly, Csaba Kari, Marc T. Abrams, Eric Wickstrom, and Ulrich Rodeck. In addition, we have monitored the activity of two other MAPKinases, i.e. JNK and p38, during suspension culture and have excluded that they play a major role in BIM phosphorylation or expression. However, we noticed strong JNK/p38 phosphorylation in suspension culture, which was only marginally affected by EGFR activation. We decided to pursue regulation of these two MAPKs in forced suspension culture based on previous reports that they exert pro- or anti-apoptotic effects in different cell systems and experimental settings (3-7). We hypothesized that disruption of cortical actin organization incurred during suspension culture may be responsible for JNK/p38 activation. Results consistent with this hypothesis were obtained when exposing attached cells to cytochalasin-D. To distinguish this effect from a general stress-induced phenomenon we proceeded to collaborate with Dr. J. Garlick at TUFTS University, Boston, MA. In efforts peripherally related to this grant we had earlier characterized HaCaT cells in which E-cadherin function was blocked by stable overexpresion of a dominant negative E-cadherin construct. This work has been submitted for publication: *A novel role for E-cadherin loss during initiation of squamous cell carcinoma through modulation of integrin-mediated matrix adhesion' by Weitian Zhang, Addy Alt-Holland, Alexander Margulis, Ulrich Rodeck, Norbert E. Fusenig and Jonathan A. Garlick.* In the course of these experiments, Dr. Garlick noticed that these E-cad deficient cells also lack cortical actin organization. This observation opened the door to examine JNK/p38 phosphorylation in the absence of cellular stress. In fact, we observed increased JNK/p38 in HaCaT cells overexpressing the dominant negative E-cadherin. These recent results are an exciting new development highlighting previously unrecognized regulation of MAPK activity by cytoskeletal organization. Currently, we are collecting more evidence in support of this notion and expect to prepare a manuscript on this topic towards the end of this year. In cooperation with Dr. Garlick we intend to use this project as the foundation of an NIH-funded research application and to revisit the functional contribution of the JNK/p38 MPKs to keratinocyte survival and death.

Specific Aim 2

As outlined in the previous progress report we have completed the work proposed in Specific Aim 2.

KEY RESEARCH ACCOMPLISHMENTS

- Completed characterization of the regulation of proapoptotic Bcl-2 family member BIM in keratinocyte suspension cultures;
- Obtained evidence for involvement of PKC in posttranslational modification of BIM in forced suspension cultures;
- Identified a novel mechanism for regulation of the MAPKinases JNK and p38 related to the organization of cortical actin cytoskeleton.

REPORTABLE OUTCOMES

A manuscript describing the results obtained under Specific Aim 2 has been published in Cancer Research. Two more manuscripts were submitted for publication during year 3 of this grant as outlined in the section describing research progress.

CONCLUSIONS

During the first 36 months of funding we have accomplished the objectives laid out in Specific Aim 2 of the original proposal. In parallel, we have now developed a strong case for posttranslational modification of BIM through EGFR activation; these results are related to Specific Aim 1 as outlined in the original proposal. In addition, we have widened the scope of our investigation to the MAPKinases JNK and p38, which are strongly phosphorylated in suspension culture. These results will be pursued with vigor for the remainder of the grant as they are related to a novel mechanism of MAPK signal transduction through actin organization.

REFERENCES

1. Kari, C., Chan, T. O., Rocha de Quadros, M., and Rodeck, U. Targeting the epidermal growth factor receptor in cancer: apoptosis takes center stage. Cancer Research., *63:* 1-5, 2003.
2. Rodeck, U., Jost, M., Kari, C., Shih, D. T., Lavker, R. M., Ewert, D. L., and Jensen, P. J. EGF-R dependent regulation of keratinocyte survival. Journal of Cell Science, *110:* 113-121, 1997.
3. Fuchs, S. Y., Adler, V., Pincus, M. R., and Ronai, Z. MEKK1/JNK signaling stabilizes and activates p53. Proceedings of the National Academy of Sciences of the United States of America, *95:* 10541-10546, 1998.
4. Cardone, M. H., Salvesen, G. S., Widmann, C., Johnson, G., and Frisch, S. M. The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. Cell, *90:* 315-323, 1997.
5. Antonyak, M. A., Moscatello, D. K., and Wong, A. J. Constitutive activation of c-Jun N-terminal kinase by a mutant epidermal growth factor receptor. Journal of Biological Chemistry, *273:* 2817-2822, 1998.
6. Smith, A., Ramos-Morales, F., Ashworth, A., and Collins, M. A role for JNK/SAPK in proliferation, but not apoptosis, of IL-3-dependent cells. Current Biology, *7:* 893-896, 1997.
7. Assefa, Z., Vantieghem, A., Declercq, W., Vandenabeele, P., Vandenheede, J. R., Merlevede, W., de Witte, P., and Agostinis, P. The activation of the c-Jun N-terminal kinase and p38 mitogen-activated protein kinase signaling pathways protects HeLa cells from apoptosis following photodynamic therapy with hypericin. Journal of Biological Chemistry, *274:* 8788-8796, 1999.