Targeting the Epidermal Growth Factor Receptor in Cancer: Apoptosis Takes Center Stage

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Abstract

Aberrant activation of the epidermal growth factor receptor (EGFR) is frequently observed in neoplasia, notably in tumors of epithelial origin. Attempts to treat such tumors with EGFR antagonists have met with remarkable initial successes, particularly when EGFR antagonists were used in combination with chemotherapy or ionizing radiation. Considering the almost ubiquitous expression of the EGFR in normal epithelial tissues, these clinical trials also revealed a surprisingly low rate of adverse side effects associated with EGFR blockade. This review highlights anti-apoptotic effects of EGFR activation as they relate to therapeutic efficacy of EGFR blockade. We introduce the concept that control of cell survival through EGFR activation is conditional in the sense that it is rate limiting to tumor cell survival but not to survival of normal epithelial cells. Specifically, normal epithelial cells are provided with a full complement of physiological cell-cell contacts and cell-matrix interactions that lessen their dependence on survival signals provided by the EGFR. By contrast, malignant tumor cells faced with inadequate cell-matrix contacts critically depend on EGFR activation for survival, rendering them more susceptible to apoptosis induction by EGFR blockade. Redundant control of cell survival by the EGFR and extracellular matrix/cell adhesion receptors is enabled, in part, by shared signal transduction pathways that control expression and activation states of members of the Bcl-2 family of apoptosis regulators.

Introduction

Multicellular organisms critically depend on efficient intercellular communication. This information exchange instructs cell fate during development, maintains homeostasis in the mature organism, and coordinates appropriate responses to challenges of the homeostatic state. Information flow is enabled by (1) extracellular matrix components binding to adhesion receptors (2), cell-cell contact between neighboring cells (3), and soluble mediators (growth factors, cytokines, hormones) that engage specific cell surface receptors. Signals emanating from matrix and cell contact receptors provide positional information as they are spatially confined. By contrast, soluble mediators can act at a distance whether by distribution through the blood stream or by diffusion. Thus, soluble factors provide a rapid and versatile response to disturbances of the homeostatic state. This is perhaps best illustrated by the wound healing process because it is characterized by successive waves of soluble mediators with complex effects on multiple cell types in the wound bed. The burst of biological activities during wound healing is limited in time and space eventually leading to the status quo ante.

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By contrast, malignant tumors frequently show constitutive deregulation of growth factor receptor signaling. In fact, among the first viral oncogenes to be described were platelet-derived growth factor B (1) and a constitutively active form of the EGFR (2) (3), the subject of this review. Deregulation of growth factor receptor signaling is not limited to virally induced tumors but occurs across a wide spectrum of spontaneous tumors of diverse tissue origin.

The EGFR Family in Epithelial Neoplasia. In epithelial malignancies, the ErbB family of receptors and their ligands are prominent targets of genetic or epigenetic alterations, which frequently lead to their inappropriate activation. The EGFR (EGFR/c-ErbB1/Her-1) is one of four members of the ErbB family of type-I-tyrosine kinases, which also includes ErbB2/Her2, Her3, and Her4. Six ligands of the EGFR are known to activate the tyrosine kinase moiety of the receptor, and the signal strength of EGFR activation may be amplified by heterodimerization with other members of the ErbB family, notably ErbB2. Epidemiological evidence accrued over the last 20 years in human tumors buttresses the notion that aberrant EGFR expression and signaling contribute to the development of multiple epithelial malignancies in humans. These include squamous carcinomas of the skin and breast cancer among others (4–9). In several epithelial tumor systems, EGFR alterations occur at advanced stages of malignancy characterized by metastatic competence (7, 10). In certain tumors, including some glioblastomas and, as shown recently, breast cancers, a truncated EGFR lacking a portion of the extracellular domain is expressed (EGFRvIII). This altered version of the EGFR appears to be constitutively active and is oncogenic in 3T3 transformation assays (11–13). In addition to the EGFR, ErbB2 is well recognized as a proto-oncogene consistent with a broad role of ErbB receptors in malignant transformation. Because of space considerations, we focus the following discussion to the EGFR/ErbB1; for in-depth reviews of the interdependent roles of ErbB1 and ErbB2 in oncogenesis, please refer to Refs. 14, 15.

EGFR Signaling as It Relates to the Malignant Phenotype. In normal or malignant skin epithelial cells, EGFR activation drives cell cycle progression (16, 17), supports migration and invasion (2, 18, 19), and affects differentiation (20, 21). Similarly, EGFR activation supports scattering and invasion of breast epithelial cells in three-dimensional culture associated with loss of cell polarization and other features of epithelial differentiation (22). Any of these effects alone or in combination may contribute to the malignant phenotype. However, in this review we will focus on EGFR-dependent cell survival as we consider this phenomenon to be critical to cancer biology and highly relevant to recent attempts to treat epithelial cancer by blocking the EGFR (for reviews of recent clinical trials see Refs. 23, 24). We will describe evidence that EGFR blockade enhances apoptosis susceptibility in conditions of cellular stress, outline molecular mechanisms...
involved in this phenomenon, and discuss the results of recent preclinical and clinical studies of EGFR blockade in this context.

**Cell Death by EGFR Blockade.** The development of EGFR specific inhibitors enabled the rigorous study of the relative contribution of EGFR signaling to cellular phenotypes. Two types of inhibitors have been produced distinguished by different modes of action. MAbs to the external domain of the EGFR have been developed that disrupt ligand binding to the receptor and subsequent signal transduction. Three EGFR-specific blocking antibodies have been characterized in greater detail in vitro and are presently used in clinical studies; these are mAbC225 (ERBITUX/ cetuximab), mAb425 (EMD72000) and the human mAb ABX-EGF. In contrast to MAbs, small molecular weight inhibitors of the EGFR block the ATP acceptor site (lysin 721) located within the intracellular kinase domain. Blockade of ATP loading prevents phosphate transfer to tyrosine residues and, thus, autophosphorylation of the EGFR and subsequent signal transduction. Examples for this type of inhibitor are EGFR selective tyrophostins (25), including the ZD1839 compound (Iressa; Ref. 24).

A role of EGFR activation in support of epithelial cell survival was first suggested by the observation that treatment with mAbC225 induces spontaneous apoptosis of the colorectal carcinoma cell line DiFi (26); the term spontaneous apoptosis is used here to describe apoptosis in the absence of obvious cellular stress. However, mAbC225 treatment rarely induces spontaneous apoptosis in malignant epithelial cells other than the DiFi cell line (23). Similarly, normal keratinocytes and squamous carcinoma cells tolerate EGFR blockade by treatment with mAb425 as long they are maintained under homeostatic culture conditions in vitro (27).

Dramatically different results were obtained when cells were subjected to cellular stress in vitro. For example, in normal keratinocytes, EGFR blockade induces large-scale apoptosis when cells are passaged (27). In addition, EGFR blockade sensitizes human keratinocytes to apoptosis induction by UV radiation (28). In the preclinical setting, mAbC225 has been shown to enhance apoptosis of malignant epithelial cells induced by many cellular stressors, including ionizing radiation and various chemotherapeutic drugs (for review see Ref. 23). Collectively, these results indicate an important survival function of the EGFR in conditions of cellular stress for both normal (e.g., primary keratinocytes) and transformed epithelial cells. Yet, they do not explain why systemic EGFR blockade is well tolerated in experimental animals or patients. Recent experiments focusing on anchorage-independent cell survival may help to resolve this issue.

**Matrix-independent Cell Survival Enabled by EGFR Activation.** Frisch and Francis (29) were among the first to realize that loss of matrix attachment leads not only to growth arrest but also to death of normal epithelial cells (immortalized keratinocytes, MDCK cells) by apoptosis and have termed this phenomenon anoikis. Other cell types, including mammary epithelial cells (30), endothelial cells (31), and some fibroblast cell lines (32), are similarly prone to anoikis when adhesion receptors are disengaged for prolonged periods of time. The dependence on matrix interaction for cell survival is believed to provide an important safeguard against inappropriate expansion and metastatic spread of normal epithelial cells. Consistent with this notion, anoikis-resistant tumor cells generally metastasize at high rates in animal models (33, 34). This leaves the question how tumor cells achieve anchorage-independent survival.

Recent work has demonstrated that activation of the EGFR provides a measure of protection against anoikis in the suspended state even to normal cells such as keratinocytes (35) or mammary epithelial cells (36). Importantly, EGFR blockade sensitizes normal epithelial cells to apoptosis induction in the suspended but to a much lesser extent in the attached state (35, 37). This observation extends to malignant epithelial cells as 6 of 7 tested squamous carcinoma cell lines die at faster rates upon EGFR blockade in the suspended but not the attached state. Taken together, these results suggest that adhesion receptors and growth factor receptors such as the EGFR provide complementary and functionally redundant survival signals to epithelial cells. Because of this redundancy, normal cells that receive the full complement of physiological matrix-derived survival signals are relatively resistant to apoptosis induced by EGFR blockade (Fig. 1A). By contrast, malignant tumor cells in transit or at sites with inadequate matrix composition have little tolerance for EGFR blockade because adhesion receptor signaling is reduced or absent (Fig. 1B). These cells are also much less likely to resist additional stress caused by either chemotherapeutic drugs or radiation therapy as compared with their normal counterparts.

It should be noted that the EGFR is but one of several receptor tyrosine kinases that are known to alleviate anoikis, including the insulin-like growth factor-I-receptor (38) and Met (39). Similarly, up-regulation of integrin-dependent signal transducers, including focal adhesion kinase (38, 40) and integrin-linked kinase (41–44) alleviate anoikis in certain tumor cells. Thus, a diverse array of kinases modulates anoikis sensitivity. Yet, among these kinases, the EGFR and its homologue, ErbB2 are most frequently deregulated in epithelial malignancies.

A series of recent studies using organotypic cultures representing breast acini further highlights the complex regulation of cell migration and cell survival by cell matrix adhesion and EGFR/ErbB2 signaling. Specifically, engagement of the β4 integrin subunit confers apoptosis resistance to immortalized breast epithelial cells in three-dimensional culture on reconstituted basement membranes (45). Conversely, disruption of integrin-dependent cell polarization renders these cells more susceptible to apoptosis induction. Forced expression of either Bcl-2 or Bcl-xL (46) or activation of c-ErbB2 (47) attenuates apoptosis of breast epithelial cells that have lost contact to basement membranes under these culture conditions. These studies are consistent with the notion that ErbB family members serve a dual role during cell invasion. Not only do they induce migration and invasion of breast epithelial cells in organotypic cultures (22), but they also sustain survival of cells that have lost positional survival signals. In this context, it remains to be determined whether the reduction in the diversity of antiapoptotic signals during migration and invasion is a consequence of epithelial-mesenchymal transitions that accompany EGFR activation in select tumor cells of epithelial origin (48). Specifically, loss of cell/cell and cell/matrix adhesion during epithelial-mesenchymal transitions may be induced by EGFR activation and necessitate increased reliance on EGFR activation for cell survival.

**Modulating Apoptosis Susceptibility by EGFR Activation: Molecular Mechanisms and Targets.** Several signal transduction pathways have been implicated in EGFR-dependent cell survival as it relates to the anchorage-independent state. Primarily, these include the RAS/RAF/MEK/MAPK cascade and STAT3-dependent signaling events.

EGFR-dependent MEK/MAPK signaling is essential to survival of normal or immortalized keratinocytes in the absence of matrix-derived signals (49). This conclusion is supported by the findings that (a) MAPK phosphorylation is markedly reduced in suspension culture, (b) EGF treatment leads to robust and sustained MAPK phosphorylation in suspension culture, and (c) inhibiting MEK by either PD98059 or a dominant negative expression construct induces apoptosis/anoikis in keratinocytes. Similarly, EGFR-dependent survival of fibroblasts (32) and mammary epithelial cells (36) appears to be mediated, in part, by MEK/MAPK-dependent signals. At least two
MAPK targets relevant for cell survival in the anchorage-independent state have been identified. We and others observed that, in keratinocytes, EGFR-dependent MAPK activation affects the balance of members of the Bcl-2 family of proteins that control apoptosis susceptibility. Specifically, EGFR-mediated MAPK activation is required for high level expression of Bcl-xL, an antiapoptotic member of the Bcl-2 family of proteins (35, 49, 50). By contrast, expression of the proapoptotic Bcl-family members Bad, Bak, and Bax is not affected by EGFR activation in this cell system. Similarly, Bcl-xL expression and survival of MDCK cells in the anchorage-independent state depend on EGFR signaling (51). Finally, EGFR-dependent Bcl-xL expression has been implicated in protection of glioblastoma cells expressing EGFRvIII against cisplatin-induced apoptosis (52, 53), although the role of MEK/MAPK signaling in this phenomenon remains to be determined. A second EGFR/MAPK-dependent survival mechanism targets the proapoptotic Bcl-2 family member BAD in mammary epithelial cells. Specifically, EGFR blockade prevents MAPK-dependent BAD phosphorylation on serines 112 and 155 (36). Phosphorylation is a prerequisite for sequestration and functional inactivation of BAD (54–56). Taken together, these results indicate that EGFR-dependent MAPK signaling targets apoptosis regulators of the Bcl-2 family not only by affecting their expression levels but also by posttranslational modification. The importance of MEK/MAPK signals in anchorage-independent tumor cell survival is underscored by the observation that pharmacological inhibitors of MEK restore anoikis sensitivity to human breast cancer cells (57).

Activation of the STAT3 provides a second well-documented EGFR-dependent survival mechanism (for review see Ref. 58). STAT3 activation contributes to expression of Bcl-xL in myeloma cells (59) and to survival and Bcl-xL expression of certain squamous cell carcinomas (60, 61). By contrast, in normal or immortalized keratinocytes, EGFR activation does not significantly contribute to STAT3 phosphorylation, and suppression of STAT3 activity by dominant negative constructs affects neither survival of nor Bcl-xL expression.

Fig. 1. Differential apoptosis susceptibility of normal epithelial tissues (A) and tumor cells during invasion and metastasis (B). In normal tissues, multiple signaling pathways converge on Bcl-2 family members to enhance protection against apoptosis induced by cellular stress. Cooperative signaling through direct cell-cell contact, interaction with extracellular matrix components, and soluble mediators provides complementary and redundant antiapoptotic signals. By contrast, cancer cells in transit or at sites with inappropriate matrix composition rely heavily on growth factors such as EGFR ligands for survival. EGFR blockade will critically weaken tumor cell resistance to apoptosis induction in inhospitable microenvironments whereas normal cells maintain a measure of apoptosis resistance through positional signals.
pressure by these cells (49). These results raise the intriguing question whether EGFR-dependent STAT3 activation is a tumor-associated event suitable to therapeutic targeting.

In certain experimental settings, EGFR activation is associated with activation of the PI3k/AKT survival pathway (62–64) or nuclear factor κB (65, 66). As discussed for STAT3, AKT/PKB phosphorylation through EGFR engagement may be restricted to certain tumor cells or experimental conditions as it is not observed in normal or immortalized keratinocytes (49). However, forced expression of oncogenic Ras (Ha-RasV12) enables EGFR-dependent AKT phosphorylation in keratinocytes in forced suspension culture (67). Similarly, forced overexpression of the tumor-associated EGFR+/variant III in fibroblasts is associated with strong activation of PI3k (13). Clearly, additional studies are necessary to distinguish physiological and tumor-associated effects of EGFR activation on signaling pathways.

Of note, EGFR-mediated and extracellular matrix-induced signaling events converge on regulating expression levels and functional states of Bcl-2 family members. For example, in CHO cells, α5/β1 integrin engagement up-regulates Bcl-2 expression in a Ras/PI3k-dependent manner (68). Furthermore, matrix adhesion prevents functional activation of the proapoptotic Bcl-2 family member Bax in mammary epithelial cells in a focal adhesion kinase and PI3k-dependent manner (69). Finally, activation of E-cadherin reportedly enhances survival of squamous carcinoma cells in a three-dimensional culture model, and this effect is paralleled the up-regulation of Bcl-2 (70). Collectively, these results reinforce the notion of redundancy in survival signals emanating from cell-cell, cell-matrix, and growth factor receptors.

Conclusions

Recent work has firmly established that EGFR activation supports survival of nontransformed and transformed epithelial cells. However, the survival function of the EGFR appears to be conditional in the sense that, particularly, tumor cells faced with inappropriate or inadequate cell-matrix contacts critically depend on EGFR activation for survival. This circumstance may explain preferential killing of tumor cells by use of EGFR blocking agents in the adjuvant setting in tumor patients. Additional work is needed to understand the coordinate and potentially redundant regulation of cell survival by activation of the EGFR, related tyrosine kinases, and cell-matrix adhesion receptors.

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References


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