Review Article

EGFR Signaling in Colorectal Carcinoma

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The epidermal growth factor receptor (EGFR) and its downstream signaling pathways are involved in the development and progression of several human tumors, including colorectal cancer. Much attention has been given to the EGFR pathway as of lately because both EGFR and some downstream components serve as targets for anticancer therapy. In addition to playing a critical role in targeted therapy, alterations in this pathway can have prognostic implications. The EGFR pathway and its impact on colorectal carcinogenesis and prognosis are the emphasis of this paper. Since prognosis is tightly related to response to various therapies, the predictive value of the components of this pathway will be briefly discussed, but this is not the focus of this paper.

1. Introduction

The epidermal growth factor receptor (EGFR) and its downstream signaling pathways regulate key cellular events that drive the progression of many neoplasms. EGFR is expressed in a variety of human tumors, including gliomas and carcinomas of the lung, colon, head and neck, pancreas, breast, ovary, bladder, and kidney. Mutations, gene amplification, and protein overexpression of various elements of this pathway not only contribute to carcinogenesis but also impact prognosis and provide specific targets for therapeutic intervention. The importance of EGFR and its signaling pathway in colorectal carcinogenesis is the topic of this paper. Since prognosis is tightly related to response to various therapies, the predictive value of the components of this pathway will be discussed, but only briefly. There is another paper in this series, “Impact of KRas mutations on management of colorectal cancer” by Sullivan and Kozuch, which provides an in-depth review of the predictive value of KRas and other members of the EGFR signaling pathway.

2. EGFR and the EGFR Signaling Pathway

EGFR is a 170-kDa transmembrane tyrosine kinase receptor that belongs to the ErbB family of cell membrane receptors. In addition to EGFR (also known as HER1 and ErbB-1), other receptors in this family include HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). All of these receptors contain an extracellular ligand-binding region, a single membrane-spanning region, and a cytoplasmic tyrosine-kinase-containing domain.

In normal cells, the EGFR signaling cascade begins with ligand activation of EGFR (Figure 1). Up to eleven ligands can bind the ErbB family of receptors, including EGF and transforming growth factoralpha [1]. Ligand binding induces dimerization of the receptor with formation of homodimers and heterodimers, which leads to the activation of tyrosine kinase. The intracellular tyrosine kinase residues then become autophosphorylated, inducing activation of multiple signal transduction pathways. Two main intracellular pathways activated by EGFR are the mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase- (PI3K-) protein kinase B (AKT) pathway. These pathways lead to the activation of various transcription factors that then impact cellular responses such as proliferation, migration, differentiation, and apoptosis [2].

Signaling through the EGFR pathway is a complex process that requires tight regulation [2]. The first level of complexity is encountered at the receptor level, where
multiple ligands are shared and lateral signaling occurs between members of the ErbB family. Then there are positive and negative feedback loops built into the pathways and differential activation of transcription factors, depending upon the cell type. When this tightly regulated system goes awry, it can contribute to malignant transformation and tumor progression through increased cell proliferation, prolonged survival, angio genesis, antiapoptosis, invasion, and metastasis [3, 4].

3. The EGFR Pathway and Colorectal Carcinogenesis (Table 1)

3.1. EGFR Protein Expression. EGFR expression (or overexpression), typically determined by immunohistochemistry, has been found to be associated with tumor progression and poor survival in various malignancies, such as carcinomas of the head and neck [5]. However, the significance of EGFR protein expression is controversial in other tumors, such as lung carcinomas [6]. Although EGFR has been reported to be overexpressed in anywhere from 25% to 82% of colorectal cancers [4], some recent studies report protein overexpression (defined as 2+ and/or 3+ staining or in >50% of cells) in 35 to 49% of cases [7–9]. However, the clinical significance of EGFR overexpression in colorectal cancer is uncertain. While one study of 249 colorectal cancers demonstrated an association of EGFR overexpression with tumor grade (poor differentiation) \( (P = .014) \) [8], another group found no association with grade in 134 tumors [9]. Similarly, some studies have found an association between EGFR overexpression (defined as 2+ or 3+ intensity) and reduced survival [7, 9], while others have not [4].

Due to the known expression of EGFR in colorectal cancer, a phase II trial of cetuximab, an anti-EGFR monoclonal antibody, in patients with refractory EGFR-positive...
The significance of EGFR gene amplification/increased EGFR copy number is more difficult to summarize. Some studies report that EGFR gene amplification (assessed by in situ hybridization methods) is uncommon in colorectal cancer [19, 20]. In contrast, in recent studies on chemorefractory colon cancers, it appears that modest increases in copy number (three- to fivefold) are present in up to 50% of cases [21]. It appears, however, that increased EGFR protein expression does not always translate into increased EGFR gene dosage [17, 18].

The wide range of EGFR expression in colorectal cancer reported in the literature, as well as the uncertain significance of EGFR expression as a prognostic indicator, may be related to the methodology used to detect EGFR. Most studies use immunohistochemistry to detect EGFR expression in colorectal cancers. As demonstrated by the experience of HER2 expression in breast cancer, immunohistochemistry is highly dependent on the antibody clone that is used, staining protocols, selection of scoring methods, and selection of cutoff values. Until a standard method of EGFR staining and reporting is adopted, the significance of EGFR protein expression in colorectal cancer remains controversial.

### Table 1: Components of the EGFR signaling pathway important in colorectal cancer.

| Component (gene/protein) | Protein function | Defect in CRC | Frequency | Prognostic | Impact Predictive (to anti-EGFR therapy) |
|--------------------------|------------------|---------------|-----------|------------|-----------------------------------------|
| EGFR/EGFR                | Transmembrane tyrosine kinase receptor | Protein expression | 25–90% | Controversial | No correlation |
|                          |                  | Mutation | Rare | Unknown | |
|                          |                  | Increased copy number | 0–50%* | Uncertain | |
| KRas/KRas                | GDP-/GTP-binding protein; facilitates ligand-dependent signaling | Activating mutation (codons 12, 13, 61, 146); leads to activation of MAPK pathway | 30–40% | Controversial | No response (if KRas is mutated) |
| BRAF/B-Raf              | Serine-threonine protein kinase downstream of KRas | Activating mutation (V600E) | 5–12% | Poor prognosis in MSS tumors | No response (if BRAF is mutated) |
| PI3CA/PI3K              | A key signal transducer in the PI3K-AKT pathway | Activating mutation (exons 9 and 20) | 14–18% | Poor prognosis in KRas wt tumors | No response (if exon 20 is mutated) |
| PTEN/PTEN               | A protein tyrosine phosphatase enzyme; inactivates PI3K pathway | Loss of protein expression; mutation; LOH | 13–19% | Poor prognosis in KRas wt tumors | No response (possibly) |

CRC: colorectal cancer; LOH: loss of heterozygosity; wt: wild-type.

*Low % for high (>10 copies) amplification; higher % for low number of copies (3–5 copies).

(assessed by immunohistochemistry) colorectal cancer was undertaken [10]. The results of this trial, reported in 2004, were promising. It was soon discovered, however, that there was no correlation between EGFR expression in the tumor and response to therapy [11, 12]. In the study by Chung et al., four of 16 (25%) patients with EGFR-negative tumors who received cetuximab-plus-irinotecan therapy achieved a partial response with a greater than 50% reduction in the size of measurable lesions [11]. This response rate is nearly identical to the 23% response rate seen in a separate cetuximab-plus-irinotecan clinical trial in EGFR-positive patients [12]. As a result, cetuximab is now administered as indicated without the need for EGFR testing.

3.2. EGFR Mutations, Gene Amplification, and Copy Number. Mutations affecting the extracellular domain of EGFR, often accompanied by gene amplification, are frequent in glioblastomas [13], while mutations in the tyrosine kinase domain of EGFR, also frequently associated with increased EGFR gene copy numbers, are clinically relevant in lung adenocarcinoma [6, 14–16]. Unlike lung cancer and other tumors, EGFR gene mutations are uncommon in colorectal cancers [17, 18].

Similarly, the predictive significance of EGFR gene amplification is also confusing and uncertain. One study of 47 patients with metastatic colorectal cancer treated with a cetuximab-based regimen showed that EGFR gene copy gain, as assessed by fluorescence in situ hybridization, had no correlation with objective response rate, disease control rate, progression-free survival, or overall survival [23]. Conversely, another study of 173 patients with KRas wild-type metastatic colorectal cancer treated with a cetuximab-based regimen found that EGFR amplification/increased EGFR copy number, present in 17.7% of patients, was associated with response to anti-EGFR therapy [24]. These conflicting results may be related to the fact that there are no established guidelines for EGFR gene amplification. But since there are no guidelines, testing for EGFR gene amplification in colorectal cancer is not routinely performed.
In addition to molecular alterations of the EGFR gene, activation of EGFR downstream effectors can lead to tumor formation/progression. Specific alterations can impact prognosis and predict response to anti-EGFR therapy.

3.3. KRas Mutations. The KRas proto-oncogene encodes a 21-kDa guanosine 5’-triphosphate (GTP)-binding protein at the beginning of the MAPK signaling pathway. Somatic KRas mutations are found in many cancers, including 30%–40% of colorectal cancers, and are an early event in carcinogenesis [25–29]. KRas mutations, most commonly codon 12/13 missense mutations, lead to constitutive activation of the KRas protein by abrogating GTPase activity. These mutations result in unregulated downstream signaling that will not be blocked by antibodies that target the EGFR receptor.

The prognostic significance of KRas mutations is controversial. KRas mutation status is associated with shorter survival in some studies [28, 30–32], but not others [29, 32, 33]. The results of one study, which showed increased mortality with codon 13 G-A mutations but not with KRas mutations in general, suggest that prognosis may be related to specific mutations in the KRas gene [28]. Although not predictive of outcome with standard chemotherapy, KRas mutation status is a strong predictive marker of resistance to EGFR-targeted therapy in patients with metastatic colorectal cancer (i.e., KRas mutations predict a lack of response to anti-EGFR monoclonal antibodies cetuximab and panitumumab) [34–39]; this topic is discussed in detail in another paper in this series, “Impact of KRas mutations on management of colorectal cancer” by Sullivan and Kozuch.

3.4. BRAF Mutations. The BRAF gene encodes a serine-threonine protein kinase that is downstream of KRas in the MAPK signaling pathway. BRAF mutations occur in 5–22% of all colorectal cancers [40, 41]. When separated by microsatellite instability status, BRAF mutations are present in 40–52% of colorectal cancers that arise through the microsatellite instability pathway (MSI) pathway (microsatellite unstable tumors) [41–44], but only 5% of cancers are microsatellite stable [42]. The most frequently reported BRAF mutation is a valine-to-glutamic acid amino acid (V600E) substitution [45]. BRAF mutations are mutually exclusive with KRas mutations [41].

Unlike KRas mutations, BRAF mutations do have an impact on prognosis and survival. In some studies, the effect is dependent upon the microsatellite status of the colorectal cancer. Patients with a BRAF mutation in a microsatellite-stable colon cancer have significantly poorer survival than those without the mutation, but the BRAF status does not affect survival of patients with microsatellite-unstable tumors [29, 42]. In patients with metastatic KRas wild type tumors, BRAF mutations have been associated with shorter progression-free and shorter overall survival [24]. BRAF status also predicts response to anti-EGFR therapy. Of metastatic colorectal cancers that are found to be KRas wild type at codons 12/13, 5% to 15% can harbor BRAF mutations and show resistance to anti-EGFR therapy [46, 47]. The predictive role of BRAF mutations is further covered in another article in this series, “Impact of KRas mutations on management of colorectal cancer.”

3.5. The PI3K Pathway-PIK3CA Mutations and Expression of PTEN and p-AKT. The PI3K-AKT pathway can be deregulated by activating mutations in the PIK3CA gene (p110 subunit), by inactivation (often by epigenetic mechanisms) of the phosphatase and tensin homolog (PTEN) gene, or by activation of AKT [1, 48]. The PIK3CA gene encodes phosphatidylinositol 3-kinase (PI3K), a key signal transducer in the PI3K-AKT pathway. Mutations in PIK3CA occur in 14% to 18% of colon cancers, and most mutations involve hotspots on exons 9 and 20 [47, 49]. Interestingly, there is a strong association between PIK3CA exon 9 mutations and KRas mutations [47]. As a prognostic marker, PIK3CA mutations are associated with shorter cancer-specific survival, but this effect may be limited to patients with KRas wild-type tumors [49]. Briefly, as a predictive marker, only PIK3CA exon 20 mutations appear to be associated with worse outcome after cetuximab [47].

The PTEN gene encodes a protein tyrosine phosphatase enzyme (PTEN) that dephosphorylates phosphatidylinositol-3,4,5 triphosphate (PIP3) and thereby inhibits PI3K function [1]. Loss of PTEN results in constitutive activation of the PI3K-AKT pathway. PTEN mutations and loss of heterozygosity (LOH) of the PTEN locus have been reported in 13%–18% and 17%–19% of colon cancers, respectively [50, 51]. It appears that loss of PTEN has prognostic value. Loss of PTEN protein expression (assessed by IHC) is associated with shorter overall survival in patients with KRas wild-type tumors [24]. It appears that there is an association with PTEN mutations/LOH with MSI status, but the current published results are conflicting [50, 51]. PTEN protein inactivation may also be a negative predictor of response to anti-EGFR therapy [22, 52].

AKT is a major downstream effector of PI3K. A recent study by Baba et al. examined the role of activated (phosphorylated) AKT expression in a large cohort of colorectal cancers [48]. They demonstrated that p-AKT expression is associated with early stage disease and good prognosis. They also showed that p-AKT expression is associated with PIK3CA mutation, as expected from their relationship in the EGFR pathway, but that the prognostic effect of p-AKT expression was independent of PIK3CA mutation. It is possible that p-AKT expression could serve as positive prognostic marker in patients with colorectal cancer.

In summary, the EGFR signaling pathway is a complex and tightly regulated process that is involved in growth, proliferation, and survival of normal cells. When this system goes awry and unchecked, it can lead to growth, proliferation, survival, and metastasis of neoplastic cells. Alterations within the EGFR signaling cascade, such as gene mutations, gene amplification, and protein overexpression, have been shown to contribute to colorectal carcinogenesis. Some alterations also portend a poor prognosis in patients with colorectal cancer. Due to the complex interaction of EGFR and its downstream regulators, the study of individual
components of this pathway often yields conflicting results, as noted in this paper. Hence, there are still many questions that need to be answered before we can fully understand the impact of the EGFR signaling pathway on colorectal carcinogenesis and the prognosis of patients with colorectal cancer.

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