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Activation of Signal Pathways and the Resistance to Anti-EGFR Treatment in Colorectal Cancer

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ABSTRACT

Colorectal cancer is the third most common cancer with a 5-year survival rate of less than 10%. It is caused by alterations of multiple signal pathways which are affected by both genetic and environmental factors. In some cases, EGFR is important in the carcinogenesis of colorectal cancer suggesting anti-EGFR therapy may be a potential treatment option. However, in other cases it is not effective, which may be related to its down-stream targeted gene mutations. KRAS is highly emphasized in the literature but other mutations like Src, PIK3CA, and BRAF may also be important. Furthermore, obesity may decrease the effectiveness of anti-EGFR treatment as it increases the risk factors for colorectal cancer. Using next-generation sequencing technology, it may be possible to identify all gene mutations in an individual with colorectal cancer. Therefore, gene mutations affecting anti-EGFR therapy in colorectal cancer patients can be identified. J. Cell. Biochem. 111: 1082–1086, 2010.

KEY WORDS: EGFR; KRAS; Src; PIK3CA; BRAF

Colorectal cancer (CRC) is the third most common cancer in the western world with a 5-year survival rate of about 10% if metastasis occurs [ACS, Cancer Facts and Figures, 2008], therefore necessitating the urgency for new treatments of this disease. It is now known that many genetic mutations and environmental factors are responsible for the carcinogenesis of CRC. Recently, obesity has been implicated as a major factor accounting for 14–35% of all CRC incidence [Calle and Kaaks, 2004; Huang and Chen, 2009]. Consequently, increased levels of obesity in the general population, have seen corresponding increases in the incidence of colon cancer. As many of these factors can increase the risks of CRC via multiple intracellular signal pathways, strategies for targeting specific molecules on these pathways have been used as potential therapeutic targets to treat CRC. Among them anti-epidermal growth factor receptor (EGFR) therapy has been applied clinically. However, its effectiveness in each specific case of colon cancer patient was reported to be different [Saltz, 2009]. Consequently only a small proportion of CRC patients have responded to this form of treatment. A better understanding of the different patient response rates will be necessary for designing effective treaments for individual patients.

MULTIPLE SIGNAL PATHWAYS IN COLON CANCER CARCINOGENESIS

Cancer is initiated and maintained by multiple intracellular signal pathways and each cancer may be dominated by several specific signal pathways [Chen and McMillan, 2007]. In CRC, many gene mutations have been identified such as *Apc*, *KRAS*, *PIK3CA*, *TP53*, and *Src* [Kinzler and Vogelstein, 1996]. These mutations activate multiple signal pathways that increase cell proliferation and cell growth and decrease apoptosis thus leading to CRC [Chen and Huang, 2009]. Obesity is also associated with an increased risk of CRC. Many factors in obesity have been identified to be responsible for this increased risk including increased blood levels of insulin, IGF-1, leptin, IL-6, TNF-alpha and decreased blood levels of adiponectin [Fenton et al., 2008; Fujisawa et al., 2008]. They also alter many signalling pathways including PI3K/Akt, STATS, MAPK, which are also activated by EGFR. Inhibition of these pathways by antibodies or small molecules has been evaluated for the treatment of CRC. However, CRC is a heterogeneous disease caused by different gene mutations and cancer risk factors, thus a specific regime may be needed for each individual CRC case.

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IMPORTANT ROLE OF EGFR AND ANTI-EGFR THERAPY

EGFR, a receptor tyrosine kinase, belongs to the HER family and thus is also called HER1/erbB-1 [Jorissen et al., 2003]. Structurally, EGFR like other HER family members HER2, 3, and 4 is a trans-membrane spanning protein containing an N-terminus extracellular ligand binding portion, a transmembrane portion and an intracellular C-terminal tyrosine kinase domain. The ligands of EGFR include EGF and TGF-beta. After ligand binding, the EGFR leads to dimerization which brings the C-terminal domain together resulting in autophosphorylation. This in turn activates multiple downstream pathways including RAS/RAF/MAPK, PI3K/Akt, and JAK/STAT3, which in turn activate proteins directly controlling cell proliferation, cell cycle, and apoptosis [Jorissen et al., 2003]. EGFR is necessary for many physiological activities to promote cell proliferation and growth. However, in many cancers EGFR signaling is unregulated. Together with increased EGFR ligand TGF-beta, the pathway is highly activated leading to uncontrolled cell growth. The activation of EGFR therefore results in cell proliferation which in turn activates tumor cell angiogenesis, metastasis, and invasiveness [Baselga, 2001].

As EGFR activation is suggested to play an important role in the carcinogenesis of CRC [Yamatodani et al., 2009], the inhibition of this pathway may be used for the potential treatment of the cancer. Two kinds of EGFR pathway inhibitors have been approved by the FDA; the tyrosine kinase inhibitors erlotinib and gefitinib and the monoclonal antibodies cetuximab and panitumumab [Baselga, 2001]. Cetuximab is a chimeric IgG1 and panitumumab is a fully humanized IgG2 [Meyerhardt and Mayer, 2005]. Both antibodies bind specifically to the extracellular ligand binding domain of the EGFR thus blocking the receptor and subsequent dimerization and phophorylation that in turn decrease EGFR down-stream signaling in this pathway. Erlotinib and gefitinib are small molecule tyrosine kinase inhibitors which act intracellularly by blocking phosphorylation at the ATP site.

In CRC, EGFR signaling is either activated by its increased ligand binding or activating mutations. Approximately 85% of CRC express EGFR [Normanno et al., 2009]. Thus, anti-EGFR agents such as cetuximab and panitumumab [Kopetz, 2007] have been deployed in the therapy of CRC with inhibition of cell growth and proliferation in a dose dependent manner [Huang et al., 2002]. The treatment can increase the cyclin-dependent kinase inhibitor p27 resulting in the accumulation of cells in the G1 phase and decreased numbers in S phase [Wu et al., 1996]. In a more recent study it was further demonstrated that cetuximab inhibited several EGFR downstream signaling pathways including RAS/RAF/MAPK, PI3K/Akt and STAT3 [Patel et al., 2009].

Indeed, the anti-EGFR treatments produced a good response in some patients. A phase III study using cetuximab showed an improved survival rate of CRC patients [Jonker et al., 2007] with similar results obtained from studies using panitumumab [Wainberg, 2006; Van Cutsem et al., 2007]. It has also been shown that anti-EGFR therapy sensitizes chemotherapy in CRC using fluorouracil, leucovorin, and oxaliplatin [Tabernero et al., 2007; Bokemeyer et al., 2009; Van Cutsem et al., 2009] whilst in other studies, it has been shown to increase the effect of radiotherapy [Solomon et al., 2003]. However, the overall response has been modest and limited to less than 20% of CRC patients [Baselga, 2001]. In addition, nearly all patients will eventually produce refractory tumors to the anti-EGFR treatment. The reason is probably that not all colorectal cancers are associated with EGFR activation. As shown in the study by Yamatodani et al. [2009] CRC cell lines S1 and WiDr, which have EFGR expression, are only moderately affected by the treatment of cetuximab while RKO which is EFGR protein negative, as detected by immunohistochemistry, is not affected by anti-EGFR therapeutics. This confirms that EGFR inhibition is not effective for patients with CRC which are caused by factors other than EGFR activation such as mutations in down-stream targets of the EGFR. It has been also shown that increased copy number of EGFR gene, detected by fluorescence in situ hybridization (FISH), is associated with a positive response to the anti-EGFR therapy [Moroni et al., 2005; Cappuzzo et al., 2008; Sartore-Bianchi et al., 2009]. Positive EGFR expression by histochemistry was initially used as an indicator for the anti-EFGR therapy. However, the method lacks specificity and can not reflect the correlation well between test and response [Gao et al., 2006].

The basic principle of anti-EGFR therapy is to inhibit the signal pathways activated by the EGFR. However even under the conditions of increased EGFR binding, if other mutations down-stream of the pathways or activation by other environmental factors that play major roles in the carcinogenesis of CRC occur, then anti-EGFR therapy will not be effective.

INEFFECTIVENESS CAUSED BY GENE MUTATIONS AND OTHER FACTORS

Gene mutations especially those down-stream of EGFR can affect the role of anti-EGFR treatment in inhibiting signal pathways that maintain cancer cell growth. Many of these mutations have been identified and their clinical significance is under investigation.

EGFR MUTATION AND POST-TRANSLATIONAL MODIFICATION

EGFR activating mutation has been associated with resistance to anti-EGFR therapy in lung cancer [Taron et al., 2005]. However, they are rare in CRC [Moroni et al., 2005]. Recently in a resistant colorectal cancer cell line DiFi5, it has been found that EGFR is post-translationally modified via protein ubiquitination and degradation, consequently, the Src signal pathway is increased for the maintenance of tumor cell growth [Lu et al., 2007].

KRAS/BRAF/MAPK PATHWAY

KRAS plays an important role in the carcinogenesis of colorectal cancer. Its activating mutation is common in colorectal cancer accounting for about 35–40% [Normanno et al., 2009]. KRAS gene mutations have also been associated with the ineffectiveness of anti-EGFR therapy and is a strong predictive marker [Lièvre et al., 2006; Benvenuti et al., 2007b; Amado et al., 2008]. A recent study has shown that the phosphorylation of the KRAS downstream proteins pERK1/2, pMEK1, pP70S6K, and pGSK3beta are increased in the activating mutation of KRAS [Perkins et al., 2010]. This suggests that

a screen for KRAS mutations would be essential to select patients for anti-EGFR therapy. It has been demonstrated that wild-type HRAS is required for the effectiveness of anti-EGFR therapy. In fact the activating mutation of KRAS has been used as a marker to exclude the application of anti-EGFR therapy as recommended by the National Comprehensive Cancer Net work and the American Society of Clinical Oncology [Allegra et al., 2009; Normanno et al., 2009; Plesec, 2009]. However, KRAS mutation cannot account for all cases. Not all patients with wild-type KRAS respond to anti-EGFR well. Therefore, other mutations downstream of EGFR could also affect its anti-EGFR effectiveness such as BRAF, Src, and PI3KCA.

BRAF is a serine-threonine kinase and is a principal effector of KRAS. Mutation of BRAF in CRC occurs at low levels with Benvenuti et al. [2007a] detecting just 12.5% of cases while Lièvre et al. [2006] detected none. Studies have suggested that it is associated with a decreased response to anti-EGFR therapy. In CRC patients, wild-type BRAF was required for a response to cetuximab or panitumumab [Sartore-Bianchi et al., 2009]. BRAF mutation has been associated with the activation of MAPK [Perkins et al., 2010] and increased MAPK signal is also related to drug resistance [Perkins et al., 2010]. Persistent activation of MAPK causes resistance to Cetuxmab [Yamatodani et al., 2009]. In KRAS mutated cases, simultaneously targeting of EGFR and MAPK increased the effectiveness of anti-EGFR therapy by cetuximab and panitumumab [Benvenuti et al., 2007b].

Src/PI3K/Akt PATHWAY MUTATION

With approximately 12% of CRC patients identified to have Src activating mutation [Irby et al., 1999; Chen, 2008] and about 80% have over-expression of Src, this pathway has been demonstrated to play an important role in colorectal cancer [Chen, 2008]. It has also been shown that a number of other factors like insulin, leptin, and IL-6 have also upregulated Src in carcinogenesis. Furthermore, the Cetuximab resistant colorectal cancer cell line DiFi5 has used Src-mediated signaling for their dependency on EGFR for cell survival [Li et al., 2009]. The use of a potent Src inhibitor PP2 abolished the resistance of DiFi5 to cetuximab. Activation of Src has also been shown to cause resistance to Cetuximab in a lung cancer cell line H226 whilst the Src inhibitor Dasatinib sentisized the cells to Cetuximab [Boerner, 2009; Wheeler et al., 2009]. Src was shown to translocate EGFR from plasma membrane to nucleus and nuclear EGFR has been associated with the resistance to anti-EGFR therapy by cetuximab both in vivo and on vitro [Li et al., 2009].

PIK3CA is also a common mutation in colorectal cancer (20%) and is oncogenic [Samuels et al., 2004]. It encodes the p110α subunit of PI3K and regulates the function of PI3K. It has also been associated with resistance to the effective treatment of cetuximab in colon cancer cell lines [Jhawer et al., 2008; Perrone et al., 2009; Sartore-Bianchi et al., 2009]. In an evaluation of 110 colorectal cancer patients, 13.6% were found to have PI3KCA mutations [Sartore-Bianchi et al., 2009]. None of these patients have responded to either cetuximab or panitimumab treatment and displayed a short progression-free survival.

PTEN is a negative regulator of PI3K. The loss of PTEN protein activates PI3K/Akt pathway and has been associated with poor prognosis. The deficiency of PTEN has also been associated with resistance to anti-EGFR therapy [Bouali et al., 2009; Perrone et al., 2009]. In CRC patients treated with cetuximab, PTEN as identified by FISH has been negatively correlated with response to treatment [Frattini et al., 2007; Laurent-Puig et al., 2009; Loupakis et al., 2009; Negri et al., 2010]. This was also demonstrated in 22 colorectal cancer cell lines screened for response to cetuximab treatment [Jhawer et al., 2008] whereby loss of PTEN expression in these cell lines were more resistant to therapy than in those expressing PTEN. Double mutations of PIK3CA/PTEN were highly resistant compared with PTEN loss only.

IGF

Insulin-IGF axis plays an important role in the carcinogenesis of colorectal cancer via both RAS/RAF/MAPK and Src/PI3K/Akt pathways [Pollak, 2008; Sridhar and Goodwin, 2009; Wolpin et al., 2009], which are also activated by EGFR. Thus, IGF-1 activation has also been associated with resistance to anti-EGFR therapy [Scartozzi et al., 2010]. In addition, crosstalk between the two pathways has been identified [Jin and Esteva, 2008; van der Veeken et al., 2009]. Inhibition of EGFR leads to activated pAkt which may be due to IGF-1. Thus inhibition of IGF-1 may facilitate anti-EGFR therapy.

OBESITY

Obesity has been associated with a poor prognosis of CRC. The cancer risk factors altered in obesity including increased blood level of insulin-IGF, leptin and IL-6, TNF-alpha could act via multiple signal pathways to cause drug resistance [Calle and Kaaks, 2004]. Each individual factor has been shown to play an important role in the carcinogenesis and prognosis for example, the IGF axis. These factors activate the carcinogenic signal pathways PI3K/Akt, MAPK, and STAT 3 via receptors other than EGFR [Huang and Chen, 2009]. Although IGF-1 has been important in the resistance to anti-EGFR therapy, other risk factors may also cause the resistance. It will be reasonable to predict that these patients will not respond well to anti-EGFR therapy. However, no study has been done to elucidate the effect of obesity on anti-EGFR therapy in CRC patients.

In summary, the role of EGFR in altered oncogenic signal pathways in a specific case may differ. It can play a dominant role in the carcinogenesis of some specific CRC patients but have no effects at all in other patients. The role of EGFR in the carcinogenesis could be correlated with response to anti-EGFR therapy. For example, if the cancer is mainly caused by the mutations down-stream of EGFR, anti-EGFR may have no effect. Although many of these mutations have been identified this is far from complete. The present screening methods have only detected KRAS which has great limitation. Thus a better screening profile is required to identify broad mutations related to resistance to anti-EGFR especially those having been studied clinically.

REMEDY: GENOME SCREENING OR COMBINATION TARGETED THERAPY

Development of new technologies in gene screening makes it possible to identify all gene mutation in an individual colon cancer patient. Recently, a new-generation of DNA sequencing methods

has been developed, which is different from the traditional Sanger approach [Shendure and Ji, 2008]. This new method allows for 10 times larger sequencing to be read with a significant decrease in costs. Thus, ineffectiveness of anti-EGFR therapy related with gene mutations in the down-stream targets of EGFR could be identified. It is possible now to individualize the use of anti-EGFR in patients with next-generation genomic sequencing technology and thus really engage in personalized medicine. In addition, the activation of signal pathways that confer the resistance to anti-EGFR therapy may be caused by other factors like obesity. Thus, detection and monitoring of signaling pathways may also be used to predict the response to anti-EGFR therapy and combining anti-EGFR therapy with other signal pathway inhibitors like Src inhibitor Dasatinib may greatly improve therapeutic effectiveness.

CONCLUSION

The ineffectiveness of anti-EGFR may be caused by several reasons including that of KRAS, Src, PI3KCA mutations, and obesity. With new rapid low cost genomic sequencing on the horizons, this should enable us to screen all gene mutations in an individual CRC patient to facilitate the design of a specific treatment regime. The incorporation of anti-EGFR therapeutics into such a treatment regime will only be effective in specific cases.

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