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TOPIC HIGHLIGHT

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Exploiting novel molecular targets in gastrointestinal cancers

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Abstract

Novel molecular targets are being discovered as we learn more about the aberrant processes underlying various cancers. Efforts to translate this knowledge are starting to impact on the care of patients with gastrointestinal cancers. The epidermal growth factor receptor (EGFR) pathway and angiogenesis have been targeted successfully in colorectal cancer with cetuximab, panitunumab and bevacizumab. Similarly, EGFR-targeting with erlotinib yielded significant survival benefit in pancreatic cancer when combined with gemcitabine. The multi-targeting approach with sorafenib has made it the first agent to achieve significant survival benefit in hepatocellular carcinoma. Efforts to exploit the dysregulated Akt/mTOR pathway in GI cancer therapy are ongoing. These molecular targets can be disrupted by various approaches, including the use of monoclonal antibody to intercept extracellular ligands and disrupt receptor-ligand binding, and small molecule inhibitors that interrupt the activation of intracellular kinases.

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Key words: Colorectal; Pancreatic; Liver cancers; Targeted therapy; Epidermal growth factor receptor; mTOR; Angiogenesis

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INTRODUCTION

Cellular proliferation, differentiation and death are regulated by a number of extracellular factors, such as hormones, cytokines and growth hormones. Interactions between extracellular stimuli and the nucleus is mediated by a complex and interconnecting network of signaling pathways[1]. This process is often abnormal in cancer cells and our understanding of these molecular events led to the identification of novel targets for therapy development. Various approaches are been used to target these dysfunctional elements, including ligand neutralization, disruption of receptor binding, and inhibition of receptor kinases and intracellular signal messengers.

A plethora of compounds are now under development that targets these aberrant processes. Almost all of these biological agents have limited single agent activity but are synergistic when combined with conventional cytotoxic agents[2]. Therefore, they are usually tested in combination with standard therapy in specific cancer types. In colorectal cancers, fluorouracil-based regimens form the backbone of therapy in both adjuvant and metastatic settings[3-5]. Likewise, gemcitabine based therapy remains the cornerstone for untreated advanced pancreatic cancer and sorafenib is likely to become the standard therapy for hepatocellular carcinoma (HCC)[6-8].

Successful targeting of angiogenesis and the epidermal growth factor pathway has made colorectal cancer a prototypical model for the development of signaling pathway-specific agents in gastrointestinal (GI) cancers[9-11]. Akt/mTOR pathway is another candidate target in anti-cancer therapies[12]. This paper will review the approaches currently used to exploit these novel targets in the development of GI cancer therapy. The review will focus specifically on colorectal, pancreatic and primary liver cancers (hepatocellular carcinoma, or HCC).

EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAY

Epidermal growth factor receptor (EGFR) is a member of the HER-family kinases, which includes EGFR, HER2, Erbb3 and Erbb4[13,14]. Upon ligand binding, EGFR homodimerizes with another EGFR or other members of the HER-family (heterodimerization), and lead to the activation of proliferative and survival signaling pathways, such as the Ras/Raf/MEK (mitogen-activated protein kinase, or MAPK) and Akt/mTOR cascades[15].

Abnormal expression or regulation of epidermal growth factors (EGF) and the receptors are implicated in the pathogenesis of many malignancies[16]. EGFR is overexpressed or up-regulated in colorectal cancers and pancreatic cancers, and is associated with early progression

and poor survival[17-22]. Similarly, EGFR is overexpressed in HCC and is associated with aggressive features with increased cellular proliferation and reduced apoptosis. *In vitro* inhibition of EGFR in HCC cell lines results in cell cycle arrest and apoptosis[23-25]. These led to the clinical development of anti-EGFR agents as single agent, or in combination therapy in view of their *in vitro* and *in vivo* synergistic activity with cytotoxic agents[26].

Cetuximab

Cetuximab is a chimeric murine/human IgG1 monoclonal antibody that blocks ligand-dependant EGFR receptor activation. The antibody has a higher affinity for the receptor than the ligands, such as EGF and transformaing growth factor (TGF-α)[27-29]. The drug is cytostatic when administered alone but highly synergistic with irinotecan in refractory colorectal cancer xenografts, leading to clinical development in irinotecan-refractory colorectal cancer patients[30,31]. In the pivotal multi-center randomized phase Ⅲ trial, 329 patients with metastatic colorectal cancer who progressed on irinotecan-based therapy were randomized to receive cetuximab alone or a combination of cetuximab and irinotecan[9]. The patients in the combination arm achieved a superior response rate of 22.9% and median time to progression of 4.1 mo compared to 10.8% and 1.5 mo in the monotherapy arm respectively. The median survival was not statistically different between the two groups.

Compared to best supportive care, metastatic colorectal cancer patients who failed multiple previous regimens achieved better overall survival, time to progression and quality of life with cetuximab monotherapy in the recent study by National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) and Australasian Gastro-Intestinal Trials Group (AGITG)[32]. In the first line setting, Cetuximab improved response rate and time to progression when administered in combination with irinotecan-based regimen (FOLFIRI) in the CRYSTAL trial[33].

The efficacy of cetuximab with oxaliplatin-based regimen (such as FOLFOX) in second- and first-line settings is being evaluated in randomized trials (the EXPLORE and OPUS trials, respectively)[34-36]. However, the addition of cetuximab to oxaliplatin based fluoropyrimidine regimens (FOLFOX or CapOx) seemed to increase the frequency of grade 3/4 adverse events, specifically gastrointestinal toxicities, rash and lethargy[37]. The role of cetuximab in adjuvant, or postoperative, setting is being studied in 2 ongoing randomized trials (PETACC-8, Intergroup 0147) in combination with oxaliplatin-containing regimens[38-40].

Cetuximab is approved by FDA in U.S. for use in patients with EGFR-expressing colorectal cancer who failed previous irinotecan-based therapy. This was due to the fact that the trials mentioned enrolled only patients with EGFR-expressing tumors, based on preclinical data suggesting the predictive value of EGFR expression for cetuximab efficacy. However, patients with EGFR-negative colorectal cancer were later found to benefit from cetuximab therapy as well, suggesting that EGFR expression level does not correlate with cetuximab response[41,42]. This is an important lesson for the development of biological agents: patient selection based on expression, or non-expression, of specific molecular markers can be faulty. Such hypothesis should be validated vigorously in well-designed clinical trials.

The side effects of cetuximab are fairly tolerable with appropriate management. Hypersensitive infusion reaction was reported in about 3% of the patients. About 75% of patients receiving cetuximab developed a mild acneiform-like rash. The development of cetuximab-related rash seemed to correlate with response but this needs to be studied further[43].

Cetuximab was evaluated in combination with gemcitabine in advanced pancreratic cancer. Despite encouraging phase Ⅱ results, the recent randomized phase Ⅲ trial (SWOG S0205) failed to confirm the superiority of cetuximab plus gemcitabine combination over gemcitabine monotherapy in this patient population[44].

Cetuximab monotherapy proved to be tolerable in patients with advanced HCC though activity was lacking in phase Ⅱ trials[27,45]. Gruenwald *et al* enrolled 32 unresectable HCC patients and 27 were evaluable. Seventy-two percent (23 of 32) had Child-Pugh Stage A cirrhosis, 25% Stage B and 3% Stage C. Previously treated patients were eligible for this trial and 44% achieved stable disease for at least 8 wk and median time to progression was 22.5 wk. The agent is been evaluated in combination with cytotoxic chemotherapy in HCC[46].

Panitumumab

Panitumumab is a fully humanized anti-EGFR monoclonal antibody that is being evaluated in metastatic colorectal cancer. The agent has the advantage of avoiding the hypersensitive reaction typical of chimeric murine proteins, such as cetuximab. In a multi-institutional phase Ⅲ trial, patients with refractory metastatic colorectal cancer were randomized to receive panitumumab plus best supportive care or best supportive care alone[47]. Eight percent (8%) of patients receiving panitumumab achieved partial response. About 90% developed the characteristic acneiform rash comparable to cetuximab monotherapy. As expected and importantly, hypersensitivity infusion reaction for the humanized monoclonal antibody was lower than that reported for cetuximab. Combination regimens containing panitunumab are been evaluated clinically.

Erlotinib

Erlotinib is an oral quinazoline that reversibly inhibits EGF receptor tyrosine kinase. The small molecule induces in vitro cell cycle arrest and apoptosis, and has *in vivo* anti-tumor effects[48,49]. Major side effects are rash and diarrhea, characteristic of this class of drug. Erlotinib was approved in 2004 by FDA in U.S. for use as single agent in previously treated non-small cell lung cancer (NSCLC) following the demonstration of survival benefit in a randomized phase Ⅲ trial (NCIC-CTG BR.21)[50]. EGFR mutations seems to correlate with the efficacy of anti-EGFR therapy in NSCLC though effort to uncover additional molecular predictors continues[51].

Among GI cancers, erlotinib is furthest along clinical development in pancreatic cancer. Gemcitabine has been

Table 1 Agents targeting EGFR pathway in GI cancers

Agents	Tumor types	Regimen	Study design	References
Monoclonal antibodies				
Cetuximab	Colorectal cancer	Irinotecan/cetuximab	Phase Ⅲ	[9]
	Hepatocellular carcinoma	Cetuximab	Phase Ⅱ	[27]
	Pancreatic cancer	Gemcitabine/cetuximab	Phase Ⅱ	[28]
	Pancreatic cancer	Gemcitabine/RT/cetuximab	Phase Ⅱ	[29]
Panitumumab	Colorectal carcinoma	Panitumumab	Phase Ⅲ	[47]
Matuzumab	Pancreatic cancer	Gemcitabine/matuzumab	Phase Ⅰ	[60]
	Colorectal cancer	Matuzumab	Phase Ⅰ	[61]
Tyrosine kinase inhibitors				
Erlotinib	Pancreatic cancer	Gemcitabine/erlotinib	Phase Ⅲ	[52]
	Colorectal cancer	CapOx/erlotinib	Phase Ⅱ	[54]
	Hepatocellular carcinoma	Erlotinib	Phase Ⅱ	[56]
	Colorectal cancer	FOLFIRI/erlotinib	Phase Ⅰ	[55]
	Pancreatic cancer	Gemcitabine/paclitaxol/RT/erlotinib	Phase Ⅰ	[62]
Geftinib	Colorectal cancer	Gefitinib/fluorouracil/oxaliplatin	Phase Ⅱ	[63]
	Colorectal cancer	Gefitinib/oxaliplatin	Phase Ⅱ	[64]
	Colorectal cancer	Gefitinib	Phase Ⅱ	[65,66]
	Hepatocellular carcinoma	Gefitinib	Phase Ⅱ	[67]
	Pancreatic and rectal cancer	Capecitabine/gefitinib/RT	Phase Ⅰ	[68]
Lapatinib	Colorectal cancer	Lapatinib	Phase Ⅱ	[59]

RT: Radiation therapy.

the standard first-line therapy for advanced pancreatic cancer in improving symptoms and survival, but not curative[6]. In the NCIC-CTG sponsored multi-institutional trial, 569 patients with untreated advanced pancreatic adenocarcinoma were randomized to receive gemcitabine plus erlotinib or gemcitabine plus placebo[52]. Intension-to-treat analysis showed longer survival in patients receiving erlotinib plus gemcitabine (6.24 mo *vs* 5.91 mo; HR 0.82, $P = 0.038$) compared to gemcitabine only. One year survival was also higher in the erlotinib-containing arm (23% *vs* 17%, $P = 0.023$). Unlike colorectal cancer, tumor EGFR expression was not a pre-requisite in this trial. There was more frequent mild grade rash, diarrhea and hematological toxicity in the combination arm but the frequency of moderate and severe toxicities were comparable in both arms. However, routine use of erlotinib and gemcitabine combination cannot be recommended in patients with advanced pancreatic cancer in view of the high cost of erlotinib[53].

Erlotinib use in colorectal cancer remains investigational. The drug showed encouraging result when used in combination with capecitabine and oxaliplatin in previously treated disease in phase Ⅱ trial[54]. The result needs to be validated in a larger randomized trial. The drug had unacceptably high rate of toxicity when combined with dose-reduced FOLFIRI in patients with metastatic colorectal cancer[55].

Erlotinib is being tested in untreated advanced HCC patients in an ongoing open-labeled phase Ⅱ trial[56]. Tumor EGFR expression is not an exclusion criteria in this trial. Interim analysis of 25 patients suggested a longer median survival among erlotinib-responding patients of 44 wk compared to 25 wk in erlotinib-non-responders. All responders developed rashes. The trial aims to accrue a total of 40 patients.

Lapatinib

Lapatinib is an interesting oral inhibitor of two tyrosine kinases: ErbB1 (EGFR) and ErbB2 (HER-2/*neu*). The agent has significant efficacy in advanced breast cancer when combined with capecitabine[57]. Both EGFR and HER-2/*neu* are co-expressed in colorectal cancer cells and simultaneous targeting of these receptors in preclinical studies enhanced apoptosis. Lapatinib is currently being tested in previously treated colorectal cancer patients[58,59].

EGFR pathway proves to be a valid target in GI cancers, especially in colorectal cancer with cetuximab and panitumumab. The small but statistically significant survival improvement by erlotinib in pancreatic cancer has been more a demonstration of "proof-in-principle" and the optimal approach to using anti-EGFR agents in pancreatic cancer still needs to be defined. Lapatinib development will hopefully shed light on whether dual-targeting of the ErbB receptor family is a successful approach in colorectal cancer (Table 1).

ANGIOGENESIS

Angiogenesis is vital to cellular growth, reproduction and development[69]. The process is often pathological in cancers, driven by an imbalance of pro- and anti-angiogenic factors in tumors[70]. The resulting tumor-induced vasculature is often leaky and dysfunctional, leading to increase interstitial pressure that impedes the delivery of both oxygen and chemotherapeutic agents[71].

VEGF-A (commonly known as VEGF) is among the first angiogenic factor discovered and shares sequence homology to the platelet-derived growth factor (PDGF) superfamily[72,73]. VEGF-A interacts with two transmembrane receptor tyrosine kinases: VEGFR-1 (Flt-1) and VEGFR-2 (KDE, Flk-1). VEGFR-2 is the primary mediator of VEGF-A and is often overexpressed in tumor vasculatures. Activation of VEGFR-2 promotes endothelial cell proliferation, survival and migration. As such, VEGFR-2 has been a major anti-angiogenic target.

VEGF over-expression and increased microvessel

density correlated with disease recurrence, metastases and survival in colorectal cancers[74-84]. Similarly, increased VEGF expression in pancreatic adenocarcinoma was also associated with poor prognosis though some studies suggest that PDGF and bFGF, instead of VEGF-A, are more important in the modulation of angiogenesis in pancreatic cancer[85-88]. HCC is highly vascular and patients with the liver neoplasm have higher serum VEGF levels than those with benign liver tumors[89-91]. In addition, increased VEGF expression following surgical resection or prior to transarterial chemoembolization correlated with poor prognosis[92-95].

As such, angiogenesis has been a focus of GI cancer therapy and can be accomplished by monoclonal antibody and small molecule tyrosine kinase inhibitor. These anti-angiogenic agents are believed to exert their anti-tumor effects by either affecting the tumor directly, inhibiting neovascularization, or enhancing chemotherapy delivery by normalizing the tumor vasculature[71,96].

Bevacizumab

Bevacizumab is a humanized monoclonal VEGF-binding antibody with anti-angiogenic properties that is the furthest along clinical development in its class. The drug was approved by FDA in U.S. for use with intravenous fluorouracil-containing regimens in patients with metastatic colorectal cancer[97].

The hint for bevacizumab efficacy in colorectal cancer in first-line setting was observed in a phase Ⅱ trial. 104 patients with metastatic colorectal cancer were randomized to receive fluorouracil and leucovorin (5FU/LV) (control arm), 5FU/LV plus "low dose" bevacizumab (5 mg/kg) and 5FU/LV plus "high dose" bevacizumab (10 mg/kg)[98]. Patients in both bevacizumab-containing arms achieved higher response rate (control: 17%; "low dose" bevacizumab: 40%; "high dose": 24%), longer time to progression and median survival (13.8 mo; 21.5 mo; 16.1 mo, respectively). Interestingly, outcome was better in the "low dose" bevacizumab arm than the "high dose" arm and was attributed partly to a higher proportion of poor risk patients in the "high dose" arm. Bevacizumab-related toxicities in this trial included thrombosis, hypertension, proteinuria and epistaxis. Bevacizumab at 5 mg/kg was thus chosen as the recommended dose for further development.

Bevacizumab was subsequently tested in metastatic colorectal cancer patients in combination with 5FU, leucovorin, leucovorin and irinotecan (IFL) in the pivotal phase Ⅲ trial. 813 patients with untreated metastatic colorectal cancer were randomized to receive IFL plus placebo (control arm), IFL plus bevacizumab 5 mg/kg or 5FU/LV plus bevacizumab 5 mg/kg[10]. IFL superseded 5FU/LV as the standard first-line regimen in U.S. by the time this trial was planned and was chosen as the control arm. The 5FU/LV plus bevacizumab arm was added as a backup since the safety of IFL plus bevacizumab was unknown. The 5FU/LV/bevacizumab arm was discontinued later during the planned interim analysis when IFL plus bevacizumab proved to be safe. The superior survival of 20.3 mo in the IFL plus bevacizumab over the IFL plus placebo arm of 15.6 mo supported the use of bevacizumab in the first-line treatment of metastatic colorectal cancer. Consistent with the earlier phase Ⅱ trial, reversible hypertension and proteinuria were more frequent with bevacizumab use. Other rare but serious side effects include gastrointestinal perforation, thrombosis and wound dehiscence.

Bevacizumab was also tested in metastatic colorectal cancer combined with oxaliplatin-based regimen in second-line setting. In the randomized phase Ⅲ trial (E3200), patients with previously treated colorectal cancer were randomized to 3 arms: FOLFOX4 plus bevacizumab, FOLFOX4 and bevacizumab only. The dose of bevacizumab chosen was 10 mg/kg[99]. The patients were not exposed to bevacizumab previously. Preliminary result showed superior survival and progression free survival in the FOLFOX4 plus bevacizumab arm. In a separate analysis, 56% of patients receiving FOLFOX4 plus bevacizumab had bevacizumab dose reduction but the survival was not significantly different from those without dose reduction[100]. Preliminary results indicate that bevacizumab is equally effective with oxaliplatin-based regimen and should be considered in second-line setting for metastatic colorectal cancer patients without previous bevacizumab exposure.

Despite the progress with bevacizumab in metastatic colorectal cancer therapy, many clinical questions remained unanswered, such as the role of continuing bevacizumab from first- into second-line setting and the synergism of bevacizumab with oral fluoropyrimidines. The combination of bevacizumab, erlotinib plus FOLFOX was examined in a phase Ⅱ trial but 40% of patients developed unacceptable toxicity and the treatment was stopped[101]. Bevacizumab is been tested with FOLFIRI in an ongoing phase Ⅱ trial involving patients with metastatic colorectal cancer[102].

The combination of bevacizumab and gemcitabine was been evaluated in pancreatic cancer. The multi-center phase Ⅱ trial demonstrated a modest partial response rate of 21% in untreated advanced pancreatic cancer patients treated with the combination[103]. Unfortunately, the combination failed to achieve survival improvement compared to gemcitabine only therapy in the subsequent phase Ⅲ randomized trial (CALGB 80303)[104]. The combination of bevacizumab with gemcitabine plus oxaliplatin (GemOx) is being evaluated in an ongoing North Central Cancer Treatment Group phase Ⅱ trial[105].

VEGF-Trap

VEGF-Trap (Regeneron) is a novel chimeric decoy receptor with higher affinity for VEGF-A than monoclonal antibodies[106]. The molecule consists of the extracellular domains of VEGFR-1 and -2 fused to the constant region (Fc) of IgG1[107]. Preclinical studies demonstrated potent anti-tumor and anti-angiogenic activities in various cancer models, prompting further clinical testing of the agent[108,109]. Phase I study of the agent in patients with advanced solid tumors showed that the agent is well-tolerated and the toxicities, including fatigue, pain, constipation and arthralgia, can be managed safely[110]. VEGF-Trap is being tested with fluorouracil-based regimens in phase I trials[111,112].

Table 2 Agents targeting angiogenesis in GI cancers

Agents	Tumor types	Regimen	Study Design	References
Monoclonal antibodies				
Bevacizumab	Colorectal cancer	Bevacizumab/IFL	Phase Ⅲ	[10]
		Bevacizumab/FOLFOX (E3200)	Phase Ⅲ	[99]
		Bevacizumab/FOLFIRI	Phase Ⅱ	[102]
	Pancreatic cancer	Bevacizumab/gemcitabine	Phase Ⅱ/Ⅲ	[103]
		Bevacizumab/gemcitabine/oxaliplatin	Phase Ⅱ	[105]
		Bevacizumab/capecitabine/RT	Phase Ⅰ	[124]
VEGF decoy				
VEGF-Trap	Solid tumors	I-LV5FU2/ VEGF-Trap	Phase Ⅰ	[111]
	Solid tumors	FOLFOX4/ VEGF-Trap	Phase Ⅰ	[112]
Tyrosine kinase inhibitors				
Sorafenib	Hepatocellular carcinoma	Sorafenib	Phase Ⅲ	[8]
	Pancreatic cancer	Gencitabine/sorafenib	Phase Ⅰ	[116]
	Colorectal cancer	Oxaliplatin/sorafenib	Phase Ⅰ	[115]
Sunitinib	Colorectal cancer	Irinotecan/cetuximab/sunitinib	Phase Ⅰ/Ⅱ	[122]
	Hepatocellular carcinoma	Sunitinib	Phase Ⅰ/Ⅱ	[121,123]

IFL: Irinotecan/leucovorin/bolus fluorouracil; FOLFOX: Oxaliplatin/leucovorin/infusional fluorouracil; FOLFIRI: Irinotecan/leucovorin/infusional fluorouracil; RT: Radiation therapy.

Sorafenib

Sorafenib (BAY43-9006) is an oral bi-aryl urea initially developed as a potent inhibitor of Raf protein[113]. The agent is also a multi-target kinase inhibitor and has significant activity against VEGFR-1, VEGFR-2, VEGFR-3 and PDGFR. As such, sorafenib is also been evaluated for its anti-angiogenic properties. The drug significantly inhibits neovascularization in colon, breast and non-small cell lung cancer xenografts in preclinical studies, marked by decreased tumor microvessel density.

Phase I trial involving patients with refractory solid tumors showed that sorafenib is fairly well tolerated. The main toxicities were diarrhea, skin rash and fatigue[114]. Downstream ERK protein was significantly inhibited at sorafenib $\geqslant$ 200 mg bid dose, indicating Raf inhibition. Partial response was observed in one (of 6) patients with HCC (400 mg bid dose) and stable disease for more than 6 mo in 6 (of 26) of colorectal cancer patients[115,116].

Sorafenib became the first agent to achieve significant survival benefit in advanced HCC in a multi-center randomized trial (SHAPR trial)[8]. 602 patients with previously untreated advanced disease with Child-Pugh Stage A cirrhosis and good performance status (ECOG PS 0-2) were randomized to receive sorafenib or placebo. Compared to the placebo arm, patients receiving sorafenib had a longer median survival (10.7 mo *vs* 7.9 mo; HR 0.69, $P < 0.01$) and time to progression (HR 0.58, $P < 0.01$). Serious side effects were similar in both groups though diarrhea and hand-foot syndrome were more frequent in those receiving sorafenib. Criticisms of the study include the generalisability of the result since majority of the patients enrolled were European and had minimal liver dysfunction. The benefit in Child's B and C patients remains unclear. Moreover, the therapy is quite costly and is a significant financial burden for most HCC patients who live in poorer developing countries. Sorafenib continues to be evaluated in HCC in combination therapy.

Sunitinib

Sunitinib (SU11248) is an oral inhibitor of VEGFR-2, PDGFR, c-kit and FLT-3. Preclinical studies showed anti-tumor activity in various malignancies, including leukemia, breast and lung cancer models[117-119]. In a phase I study, the recommended dose for sunitinib was determined to be 50 mg/d on a "4-wk-on/2-wk-off" schedule[120]. The toxicities include hypertension, thrombocytopenia, neutropenia, diarrhea, hair and skin changes. Sunitinib is being tested in HCC and in combination with irinotecan and cetuximab in previously treated metastatic colorectal cancer[121-123].

Of the anti-angiogenic agents discussed, bevacizumab proved to be an exceptionally efficacious agent in colorectal cancer when combined with conventional cytotoxic agents. However, this monoclonal antibody failed to achieve the clinical benefit expected in pancreatic cancer in combination therapy. More excitingly, sorafenib becomes the first chemotherapeutic agent to achieve significant clinical benefit in HCC (Table 2).

AKT/mTOR PATHWAY

The mammalian target of rapamycin (mTOR) is a cytosolic serine/threonine kinase that plays a central role in cell proliferation and survival[125]. The kinase is downstream to the phosphatidylinositol 3'-kinase (PI3K)/Akt signaling pathway. Activated mTOR interacts with downstream effectors, such as 4E-BP1 and p70s6K, to modulate various growth and survival-related cellular functions. The pathway is sensitive to extracellular growth factors (EGF, VEGF and IGF) and nutrients (amino-acids, glucose and oxygen).

In a series of 101 resected primary hepatoma (with 73 HCC), 15% had overexpression of phospho-mTOR and 5% had increased total mTOR protein expression[126]. In pancreatic cancers, more than 90% of the tumors contain an activating upstream ras mutation and about half of the surgically resected pancreatic cancer specimens had mTOR activation[127-131].

Loss of the suppressive PTEN gene expression, PI3K gene mutations and amplification of Akt result in constitutive activation of the upstream PI3K/Akt pathway

observed in some tumors[126-129,132-135]. Such activation increases the tumors' susceptibility to mTOR inhibitors and provided the rationale in developing rapamycin (mTOR inhibitor) analogs in various cancer types[136-140]. In addition, inhibition of mTOR reversed gemcitabine resistance in gemcitabine-resistant pancreatic cancer cell lines in preclinical xenograft model[131]. These preclinical data support the clinical testing of mTOR inhibitors in HCC and pancreatic cancer.

Rapamycin

Rapamycin (sirolimus) is an oral macrolide derived from *Streptomyces hygroscopicus* that is widely used as immunosuppressant in organ transplantation[141-145]. Rapamycin and its analogs also inhibit cellular proliferation in a wide range of human tumors. The drug complexes with FKBP12, a member of the immunophilin family of FK506-binding proteins, intracellularly which in turn inhibits the mTOR kinase activity, leading to G1 phase cell cycle arrest and apoptosis[146,147]. However, the drugs poor aqueous solubility, chemical stability and lack of investor interest impeded its clinical development as an anti-neoplastic agent[12]. Currently, rapamycin is being tested in a pharmacodynamic-guided dose-finding study involving patients with advanced solid tumor and also in a phase Ⅱ trial involving patients with advanced pancreatic cancer[148].

Temsirolimus

Temsirolimus (CCI-779) is a water-soluble synthetic rapamycin ester with significant anti-proliferative properties that can be administered *via* both oral and intravenous routes[149-154]. The drug demonstrated comparable *in vitro* anti-tumor effect to rapamycin against a wide range of human cancer cell lines, including prostate, breast, small-cell lung carcinoma, melanoma, glioblastoma and T-cell leukemia. The agent inhibits tumor growth, or is cytostatic, in a variety of cancer xenograft models but did not achieve tumor shrinkage.

Two dosing schedules of temsirolimus were tested in separate phase I trials: weekly intravenous dose versus the 30 minute intravenous infusion administered daily for 5 d on a bi-weekly schedule[155,156]. Toxicities observed include skin changes, muscostomatitis, asthenia, myelosuppression (thrombocytopenia, neutropenia), dyslipidemia and elevated liver enzymes. Dose escalation for the weekly regimen was stopped at 220 mg/m^2, which was the highest planned dose. Toxicities were fairly manageable and reversible at this dose. Interestingly, tumor shrinkages (partial and minor responses) were observed clinically, contrary to the cytostatic phenomenon seen in preclinical studies. Two patients achieved partial response: one with renal cell carcinoma and another with breast cancer. This led to further testing of temsirolimus in various cancer types[157-160]. Temsirolimus was recently approved by FDA in U.S. for the treatment of poor risk renal cell carcinoma patients based on the positive result from a randomized phase Ⅲ trial[161].

Everolimus

Everolimus (RAD001) is an oral rapamycin analog that inhibits tumor growth and angiogenesis in a dose-dependant manner and has anti-proliferative activity against a wide range of human cancers[162,163]. The optimal biologically active dose of everolimus was studied in two phase I trials. Everolimus 20 mg weekly was determined to be biologically active and toxicities associated with weekly everolimus administration were well tolerated and included anorexia, fatigue, rash, mucositis, headache, hyperlipidemia and gastrointestinal disturbance. The dose-limiting toxicities of daily everolimus were stomatitis, neutropenia and hyperglycemia. Pre-treatment and during-treatment tumor biopsies were done to evaluate pharmodynamic effects of everolimus and a 10 mg daily dose was recommended as the optimal dose. Partial response was seen in one colorectal cancer patient and everolimus is in phase Ⅱ development as single agent in refractory colorectal cancer[164]. The agent is being developed in other cancer types as well, such as gastrointestinal stromal tumor, neuroendocrine tumors, renal cell carcinoma, non-small cell lung cancer and melanoma[165-169].

The Akt/mTOR pathway seems to be an important survival and pro-growth pathway in GI cancers. Temsirolimus is the first of its class to achieve significant anti-tumor efficacy and clinical development of the class of mTOR inhibitors in pancreatic cancer and HCC continues.

CONCLUSION

Angiogenesis and EGFR pathways were hypothesized as targets for anticancer therapy more than three decades ago. Efforts to translate this knowledge to bedside are just starting to benefit patients with GI cancers. Successful development of cetuximab and bevacizumab in colorectal cancer ushered in the era of biologically targeted agents in the fight against GI cancers. More milestones were later achieved when the survival of previously difficult-to-treat GI cancers were improved by these novel biological agents, as in the case of erlotinib in pancreatic cancer and sorafenib in HCC. More molecular targets will become apparent as our knowledge of the complex neoplastic processes increases, and will provide exciting translational opportunities in the development of GI cancer therapy.

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