Prevalence of KRAS, BRAF, NRAS, PIK3CA and PTEN alterations in colorectal cancer: analysis of a large international cohort of 7,186 patients

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Abstract

Background
Colorectal cancer (CRC) is the third most common cancer worldwide, with metastatic disease accounting for 40 to 50% of newly diagnosed patients. EGFR monoclonal antibodies (Mab), cetuximab and panitumumab, are effective treatment for KRAS wild type CRC. Although mutations in KRAS predict resistance to EGFR Mab therapy, only 80% of CRC patients with KRAS wild type status respond to treatment. This study is a retrospective evaluation of genomic alterations in the EGFR pathway such as alterations in KRAS, BRAF, NRAS, PIK3CA and PTEN that may predict lack of response to EGFR Mab therapy in CRC patients.
Method
A large database of 7,186 consecutive CRC patients was analyzed from 2011 onwards for demographics (sex, age, geography, site of tumor) and biomarkers that might correlate with response to EGFR Mab therapy. Comprehensive testing included gene sequencing for KRAS, BRAF, NRAS, HRAS, Her2, Her4, PTEN and PIK3CA (Next Generation Sequencing, Sanger, pyrosequencing) and immunohistochemistry for PTEN protein expression.
Results
Analysis revealed the incidence of KRAS mutation of 44% which is consistent with published literature. Analysis of the KRAS wild type cohort revealed a mutation rate of 16.2 % for BRAF, 7.7% for NRAS, and 42% for patients with either PTEN loss of expression/mutation or a PIK3CA mutation. Earlier studies support that mutations in NRAS, BRAF and activation of the PI3K pathway by PTEN/PIK3CA analysis result in lower response rates to EGFR Mab therapy in CRC. Furthermore, multiplex biomarker testing revealed the rare occurrence of concurrent mutations in 43 cases.
Conclusion
This is a comprehensive analysis of a large international cohort evaluating the prevalence of predictive molecular aberrations suspected of lack of response to EGFR Mab therapy in patients with wild type KRAS and their demographics. Prospective controlled studies are in progress to validate the role of BRAF, NRAS, PIK3CA and PTEN in clinical management of CRC. It is imperative to further explore the molecular pathology of CRC beyond KRAS in patient selection for EGFR Mab therapy.

Background

The Kirsten rat sarcoma viral oncogene homolog (KRAS) encodes a GTPase downstream of EGFR. It signals through the PI3K/AKT/mTOR, STAT and RAF/MEK/MAPK pathways, involved in cell survival and proliferation. Point mutations especially at position 12, 13 or 61 in exon 2 lead to an impaired GTPase activity which results in constitutive activation of RAS signaling. Mutations in the KRAS oncogene are an early event in the pathogenesis of CRC and are associated with a worse prognosis. In CRC, KRAS analysis to predict the efficacy of EGFR monoclonal antibody-targeted therapies is established in clinical practice, and KRAS mutation has been confirmed to predict a limited therapeutic benefit for patients treated with EGFR targeted monoclonal antibodies like cetuximab or panitumumab. Although KRAS testing has facilitated the selection of patients who are most likely to have a response to anti-EGFR therapy, a substantial fraction of patients do not benefit from treatment. The study hypothesis is based on further refinement of tumor specific genetic markers to allow more accurate selection of patients who are likely to have a response to a anti EGFR therapy and prevent toxic effects in those who are unlikely to benefit. **In this study the biomarker exploration has been broadened to include EGFR pathway aberrations including NRAS, HRAS, BRAF, HER2, PIK3CA and PTEN in addition to KRAS.**

Methods

All colorectal tumor cases referred to Caris Life Sciences between 2009 through Sep. 2013 from 50 states and 30 countries were evaluated; diagnoses were collected from referring physicians and classified at intake based on pathology and clinical history. Specific testing was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (immunohistochemistry), and/or gene amplification (CISH or FISH) testing.

Results

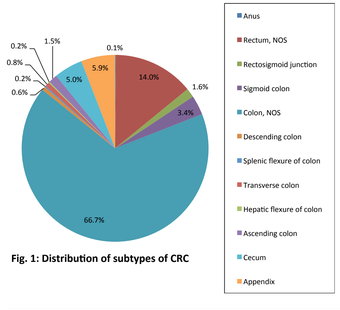

Fig. 1: Distribution of subtypes of CRC

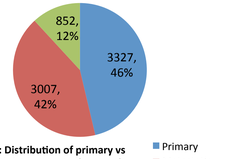

Fig. 2: Distribution of primary vs metastatic tumors in the sample analyzed

Female	3,349	20-94 yrs
Male	3,837	20-101 yrs
Total	7,186	

	IHC Her2	IHC PTEN loss	Her2 Amp.	PIK3CA Amp.	BRAF mut	EGFR mut	ERBB2 mut	ERBB4 mut	HRAS mut	KRAS mut	NRAS mut	PIK3CA mut	PTEN mut
KRAS mutated cohort													
positive	9	836	20	1	9	9	6	4	2	2758	0	136	11
total	1765	2059	692	7	2031	416	376	382	336	2758	606	658	364
%	0.5	40.6	2.9	14.3	0.4	2.2	1.6	1	0.6	100	0	20.7	3
KRAS Wildtype cohort													
positive	74	1131	74	1	460	4	8	2	0	0	59	72	13
total	2724	3175	961	12	2840	489	453	455	404	3526	764	820	445
%	2.7	35.6	7.7	8.3	16.2	0.8	1.8	0.4	0	0	7.7	8.8	2.9

Table.1: Distribution of genetic aberrations in KRAS mutated and wildtype CRC cases. NRAS and HRAS mutations were exclusive to KRAS WT and KRAS mut cohort respectively. There was statistical difference in the PIK3CA mutation frequency between the KRAS mutated (20.7%) vs KRAS WT(8.8%) cohort (p<0.0001). Similar trends were noted for PIK3CA gene copy number, however the sample size was too low to draw any conclusion. Distribution of HER2 protein and HER2 gene copy number was significantly higher in the KRAS wildtype cohort as compared to KRAS mutated (p<0.0001). This may allow activation of downstream signaling events when cetuximab is bound to EGFR, which in turn could lead to drug resistance. Data indicates that a major fraction of KRAS WT patients with activated PI3K pathway due to either loss of expression or mutation in PTEN or mutation or amplification of PIK3CA gene which could lead to impaired responses to EGFR monoclonal antibodies. NRAS mutations were found only in the KRAS WT cohort which validates the exclusiveness of this event. BRAF mutations were significantly higher in the KRAS WT cohort as compared to the KRAS mutated cohort (p<0.0001). There were 9 patients that harbored a coexisting BRAF and KRAS mutation which is an extremely rare event and indicates very poor prognosis.

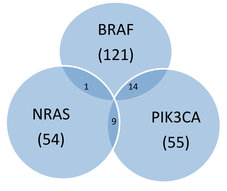

Fig. 3: Coincidence of BRAF, NRAS and PIK3CA mutation in the KRAS WT CRC patients.

Alterations in the total CRC population	
KRAS mut	44%
PTEN loss of expression	36%
PTEN mut	2.9%
BRAF mut	10%
PIK3CA mut	14.0%
PIK3CA GCN	9.5%
HER2 GCN	6%
HER2 mut	2%
HER2 expression	2%
ERBB4 mut	0.7%
NRAS mut	4.3%(exclusive to KRAS WT)
HRAS mut	0.3%(exclusive to KRAS Mut)
EGFR mut	1.4%

Percent KRAS mutation by primary site	
CRC	44%
Appendix	49%
Rectum	43%
Ascending colon	43%
Descending colon	23%

Mutation patterns in KRAS, BRAF and NRAS in CRC patients

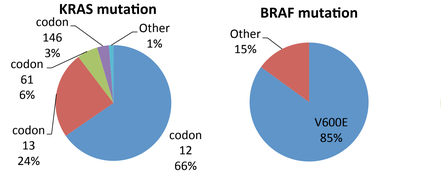

Fig. 4:KRAS mutation was identified in 44% of CRC cases while 56% specimens were determined to be KRAS wildtype. The frequency of KRAS mutation was highest in codon 12.
BRAF mutation was identified in 10% of total CRC population with majority being V600E.
NRAS mutation was identified in 7.7% of total CRC population with the frequency distribution being similar in codon 12 and 13.

Sex	Age	Primary Tumor Site	ISH Her2	BRAF Mut	PIK3CA Mut	NRAS Mut	ERBB2 Mut	PTEN Mut	Clinical History
F	62	Cecum	Not Amp.	V600E	E542K	WT	WT	WT	Colon cancer.
M	65	Cecum	Not Amp.	V600E	E542K	WT	WT	WT	Cecum adenocarcinoma.
F	76	Transverse colon	Not Amp.	V600E	E545G	WT	WT	WT	Colon cancer.
F	42	Colon, NOS	Not Amp.	V600E	E545K	WT	WT	WT	Metastatic colonic adenocarcinoma.
F	74	Cecum	Not Amp.	V600E	E545K	WT	WT	WT	Metastatic colonic adenocarcinoma.
M	57	Ascending colon	Not Amp.	V600E	E545K	NP	NP	NP	Metastatic mucinous; ascending colon.
M	28	Colon, NOS	Not Amp.	V600E	Q546R	WT	WT	WT	Metastatic colon cancer.
M	48	Colon, NOS	Not Amp.	V600E	Q546R	WT	WT	WT	Metastatic carcinoma.
F	60	Rectum, NOS	Not Amp.	V600E	H1047R	WT	NP	NP	Adenocarcinoma.
F	59	Rectosigmoid junction	Not Amp.	V600E	H1047R	WT	WT	S294R	Adenocarcinoma oF the colon.
F	79	Cecum	Not Amp.	V600E	H1065Y	WT	WT	WT	Metastatic carcinoma.
F	72	Colon, NOS	Not Amp.	V600E	N1044K	WT	WT	WT	Colon cancer.
F	71	Cecum	Amp.	V600E	NP	NP	NP	NP	Metastatic colorectal adenocarcinoma.
F	90	Colon, NOS	Not Amp.	V600E	WT	WT	WT	L265Fs	Colon cancer.
F	79	Cecum	Not Amp.	V600E	WT	WT	WT	D268Fs	Metastatic adenocarcinoma.
M	51	Splenic Flexure oF colon	Amp.	V600E	WT	WT	WT	WT	Invasive adenocarcinoma.
M	79	Colon, NOS	Not Amp.	V600E	WT	WT	WT	T319Fs	Invasive adenocarcinoma.
M	53	Colon, NOS	NP	V600E	WT	WT	WT	H196Fs	metastatic adenocarcinoma
M	82	Descending colon	Not Amp.	V600E	WT	WT	D880A	WT	Adenocarcinoma oF the colon.
F	74	Descending colon	Not Amp.	V600E	C420R	WT	WT	WT	Colon cancer.
M	78	Colon, NOS	NP	V600E	R108H	WT	WT	WT	Metastatic adenocarcinoma.
M	78	Colon, NOS	Amp.	V600E	NP	NP	NP	NP	Metastatic colonic adenocarcinoma.
F	79	Colon, NOS	Amp.	V600E	NP	NP	NP	NP	Metastatic colonic adenocarcinoma.
F	78	Rectum, NOS	NP	WT	E542K	Q61L	NP	NP	Infiltrating adenocarcinoma.
M	53	Colon, NOS	NP	WT	E545K	Q61K	WT	WT	Invasive adenocarcinoma.
F	54	Colon, NOS	Not Amp.	WT	Q546K	Q61K	WT	D187Fs, F241Fs	Metastatic adenocarcinoma.
M	41	Colon, NOS	Not Amp.	WT	E542K	G12D	T862A	WT	Metastatic colonic adenocarcinoma.
M	81	Colon, NOS	NP	WT	E545K	G12D	WT	WT	Adenocarcinoma.
F	59	Colon, NOS	Not Amp.	L597R	E542Q	WT	NP	NP	Carcinoma with neuroendocrine features.
M	26	Colon, NOS	NP	NP	E542K	WT	NP	E7X	Metastatic adenocarcinoma.
F	79	Cecum	Not Amp.	WT	Q546P	WT	WT	T319Fs	Medullary carcinoma.
M	79	Colon, NOS	Amp.	WT	Q546P	NP	NP	NP	Colon cancer.
F	41	Colon, NOS	Not Amp.	WT	E545A	WT	WT	K267Fs	Metastatic colonic adenocarcinoma.
M	80	Colon, NOS	NP	WT	H1047R	Q61K	NP	NP	Metastatic colorectal adenocarcinoma.
F	77	Colon, NOS	NP	WT	H1047R	G12D	WT	WT	Metastatic colonic adenocarcinoma.
M	66	Rectosigmoid junction	Amp.	WT	H1047R	WT	NP	NP	Colon cancer.
M	64	Sigmoid colon	Amp.	WT	G1049R	NP	NP	NP	Adenocarcinoma.
F	60	Colon, NOS	Not Amp.	WT	G106V	Q61R	WT	WT	Colonic adenocarcinoma.
M	41	Colon, NOS	Not Amp.	G469V	WT	G12C	NP	NP	Adenocarcinoma.
M	37	Cecum	NP	L584I	WT	WT	WT	E7X	Invasive colonic adenocarcinoma.
F	61	Colon, NOS	Amp.	G469R	WT	WT	NP	NP	Metastatic adenocarcinoma.
M	48	Transverse colon	Not Amp.	WT	L92F, C420R	WT	WT	WT	Adenocarcinoma.
F	72	Colon, NOS	Amp.	WT	WT	WT	WT	V777L	Metastatic colon adenocarcinoma.

Table 2: KRAS wlidtype cases with more than one genetic mutation in the EGFR pathway. A total of 43 cases were identified with co-mutations. Majority of co-mutated cases had BRAF V600E mutation (23 out of 43) highlighted yellow. Eight of those cases had an exon 9 highlighted blue and 4 had exon 20 PIK3CA mutation highlighted pink). NRAS mutations at codon 61 highlighted orange and codon 12 highlighted light green co-existed with a PIK3CA mutation. Of note, 5 out of 10 cases had presence of dual PTEN highlighted green and PIK3CA mutation.

Conclusions

1. To our knowledge this is the first study involving a large cohort of CRC cases (7,186) in which molecular alterations in the EGFR signaling pathway have been investigated in a single clinical laboratory with standardized testing. The genomic alterations investigated include frequency distribution of mutations in KRAS, NRAS, HRAS, BRAF, PIK3CA, ERBB4 and HER2 mutation/amplification, and loss of expression as well as mutation in PTEN in KRAS wildtype CRC patient samples. Our data identified 21% of KRAS WT CRC patients with no aberrations in the EGFR pathway which would make them ideal candidates for EGFR Mab therapy.
2. Low response rate to EGFR monoclonal antibody treatment in KRAS wildtype CRC patients could be attributed to the high frequency of alteration in the EGFR signaling pathway as indicated by this study. Testing for additional molecular alterations in KRAS WT CRC patients using various testing methodologies has the potential to identify those that are not likely to respond to anti-EGFR therapy alone but may respond better to combination of targeted agents based on their biomarker profile.
3. Frequency of KRAS mutation is lower in descending colon as compared to other sites.
4. Comprehensive molecular analysis of KRAS WT CRC cases has identified 43 unique patients with multiple genetic alterations which could indicate potential for targeted therapy using a combination of agents.