Molecular Profiling of Small-Bowel Adenocarcinomas

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Abstract

Background: Small-bowel adenocarcinoma (SBA) is a rare malignancy with limited knowledge of the molecular mechanisms or clinical evidence-based guidelines for therapy. We conducted a comprehensive analysis of biomarkers with therapeutic relevance for SBA.

Methods: We examined the biomarker profiles of 264 SBA cases. Multiplatform biomarker panel included a combination of sequencing (Sanger, NGS), protein expression (IHC) and gene amplification (CISH /FISH).

Results: Several biomarkers have been identified by IHC evaluation in ~250 patients: high TOP2A (74%) and low TUBB3 (81%), low ERCC1 (71%), low RRM1 (69%) and low TS (63%)- favorable for chemotherapy drugs such as doxorubicin, taxanes, oxaliplatin, gemcitabine and capecitabine, respectively. A minority of cases were found to express other favorable chemotherapy response biomarkers: low MGMT (alkylating agents, 30%), high SPARC (nab-paclitaxel, 36%) and high TOPO1 (irinotecan, 39%). SBA demonstrated a high prevalence of multidrug resistance gene expression: MRP1 and BCRP (83%) and PGP (51%). Unexpectedly, a number of cases showed genomic alterations (gene amplifications) associated with activated oncogenic signaling: EGFR (13% or 12/96), TOP2A (12% or 4/33), HER2 (7% or 9/130) and cMET (1.4% or 1/71). Two patients demonstrated co-amplification of TOP2A and HER2, suggesting these patients may benefit from anthracyclines or anti-HER2. Evaluation of mutational hotspots in 46 genes demonstrated a high incidence of oncogenic/tumor suppressor mutations in 60% (47/78) of patients. We discovered that mutations in TP53 (51%) and KRAS (46%) were most common. Other genes with notable alterations included: APC (22%), SMAD4 (20%), BRAF (9%), CTNNB1 (7%) and PTEN (5%). Recent data suggest favorable responses to cetuximab in SBA; their results and high frequency of KRAS mutations in our series indicate that KRAS testing may be recommended in SBA.

Conclusion: Our data demonstrate the potential utility of a wide range of traditional chemotherapies for SBA, targeted therapies utilized for other cancer types as well as therapies under clinical investigation. SBA exhibits biologically similar tumorigenesis as colorectal adenocarcinoma, therefore similar treatment guidelines and biomarker testing strategies should be considered.

Background

Malignancies of the small bowel are among the rarest types of cancer, accounting for only 2% of gastrointestinal (GI) cancers. Adenocarcinomas are the most common subtype of small intestinal tumors, and an estimated ~3,000 people will be diagnosed with the disease in 2013 (American Cancer Society). Delays in diagnosis of SBA is common with a majority of patients (>60%) presenting at Stage III or IV disease.

Small-bowel adenocarcinomas share a striking resemblance to large-bowel (colorectal) adenocarcinomas, including similarities in molecular pathogenesis such as high prevalence of KRAS and p53 mutations. However, this disease is lacking in approval of targeted therapies and its own unique evidence-based guidelines by the National Comprehensive Cancer Network.

Molecular profiling analysis in a cohort of 264 patients, including 55 patients with next generation sequencing data, was investigated to identify potential targeted agents that could be used for the treatment of a disease that lacks treatment guidelines.

Methods

Two hundred sixty four SBA cases referred to Caris Life Sciences from 2009 through 2013 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 (next-generation sequencing [NGS]), protein expression (immunohistochemistry) and gene amplification (CISH or FISH).

Results

Figs. 1-3. Tumor Attributes and Patient Demographics

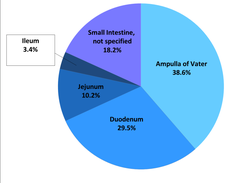

Figure 1. Tumor Location – 264 SBA were studied and grouped according to primary tumor site location.

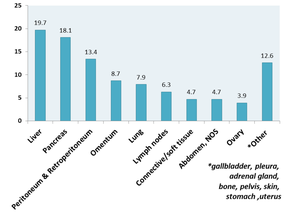

Figure 2. Sites of Metastasis – 48% of specimens studied were from metastatic sites (n=127). The most common metastatic site was the liver.

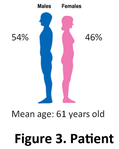

Figure 3. Patient Demographics

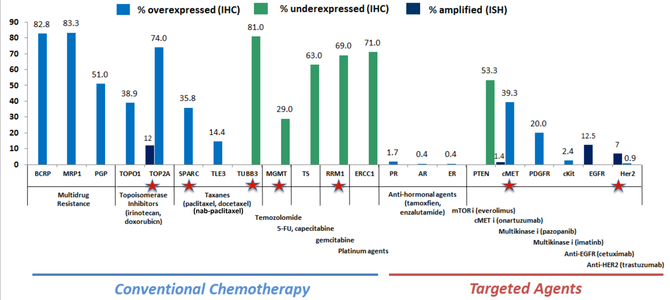

Figure 4 – Protein and gene copy number changes identified by CMI profiling and associated therapies with potential benefit. Currently, NCCN recommends SBA may be treated with systemic chemotherapy according to the NCCN guidelines for colon cancer. Theranostic markers for conventional colon cancer treatments have been tested, demonstrating potential benefit, including 39% overexpression of TOPO1, and underexpression of TS and ERCC1 (63% and 71%, respectively). ★ Novel alterations and associated therapies were identified for subgroups of patients: high cMET in 39%, low MGMT in 29%, low RRM1 in 69%, amplification of HER2 and TOP2A in 7% and 12% of patients, respectively, (including 2 patients with co-amplification) and low TUBB3 in 81% and high SPARC in 36% (of interest, 37% of low TUBB3 expressers have high expression of SPARC). Based on these results, one may consider cMET inhibitors, temozolomide, gemcitabine, anti-HER2 antibodies, anthracyclines and taxanes such as nab-paclitaxel. Many of these agents are in clinical trials for colon cancer or SBA (nab-paclitaxel: NCT01730586, tivantinib: NCT01892527) or have been reported in the literature as having some beneficial effect in colon cancer patients (HER2/trastuzumab: Sorscher, et al. 2011, MGMT/temozolomide: Shacham-Shmueli, et al. 2011, RRM1/gemcitabine: Overman, et al. 2009).

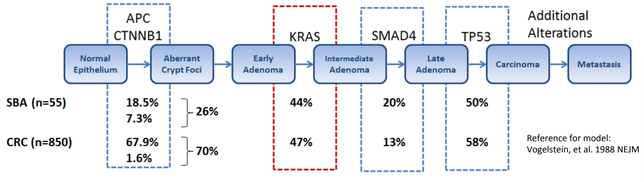

Figure 5. Mutation rates in SBA and CRC according to the genetic model of colorectal carcinogenesis. Frequencies are from tumors at late stages. CRC frequencies are derived from internal CMI data. Wnt activation (APC/CTNNB1) occurs at a much lower frequency compared to CRC. SMAD4 mutations are more frequent in SBA vs. CRC. KRAS mutation rates and spectrum observed in SBA is comparable to CRC. In the classic adenoma-carcinoma sequence, KRAS is the only predictive biomarker; when mutated, it is associated with a lack of response to cetuximab. Response to cetuximab has been reported in a series of KRAS WT SBA patients (Santini, et al. 2010).

	KRAS WT (n=31)	KRAS MT (n=24)	Associated Therapies
NRAS (NGS)	0%	0%	Modulators of cetuximab response PIK3CA inhibitors
PTEN loss (IHC)	54%	24%	
BRAF V600E	0%	0%	
PIK3CA (NGS)	3%	15%	
cMET (IHC)	32%	69%	cMET inhibitors (tivantinib)
PTEN (NGS)	3%	4%	PIK3CA, mTOR inhibitors
TP53	48%	46%	Chk1, Wee1 inhibitors
**BRAF (non-V600E)	10%	8%	BRAF inhibitors if mutation is activating (sorafenib), MEK/ERK inhibitors
ATM (NGS)	7%	0%	platinum agents
cKIT (NGS)	0%	4%	multikinase inhibitors (imatinib)
cMET (NGS)	3%	4%	cMET inhibitors (PF-04217903)
EGFR (NGS)	3%	4%	Anti-EGFR or pan-HER (erlotinib, afatinib)
HER2 (NGS)	7%	0%	Anti-HER2 or pan-HER (neratinib, afatinib)
FBXW7 (NGS)	0%	8%	mTOR inhibitors (everolimus)
GNAS (NGS)	0%	4%	MEK inhibitors
GNA11 (NGS)	9%	0%	MEK inhibitors
VHL (NGS)	4%	0%	anti-angiogenics

Table 1. Modulators of cetuximab response and additional therapy options for KRAS WT/MT SBA. Patients with NGS data were studied for this subgroup analysis (n=55). 56% of SBA patients may potentially benefit from cetuximab, based on KRAS WT status, however, over half exhibit loss of PTEN by IHC, which may reduce response to cetuximab based on evidence in colorectal cancer (Laurent-Puig, 2009). Mutations in NRAS and BRAF V600E were absent, and 1 patient harbored a PIK3CA mutation in the KRAS WT group. Therefore, the known modulators of response to cetuximab are relatively infrequent in SBA. Of interest, non-V600E mutations (**) occurred in KRAS WT and MT SBA patients.

BRAF Protein Change	Other tumor types where variant detected (internal data)	Clinical Relevance
D594N Exon 15	Breast, NSCLC, Melanoma, Ovarian	Kinase dead mutation, utility of BRAF inhibitors are not supported, however, if found in the presence of activated RAS, then MEK i are appropriate (Heidorn, S., et al. 2010)
G466E Exon 11	CRC, NSCLC, Melanoma, Ovarian	lower kinase activity than BRAF^{WT}, however still activates MEK-ERK through CRAF (Kamata, T., et al. 2010)
G469V Exon 11	CRC, NSCLC, Melanoma	Slightly higher levels of BRAF signaling vs. BRAF^{WT}, however vemurafenib lacks activity against this alteration (Yang, et al. 2010)
T599A Exon 15	n/a	Lower kinase activity than BRAF^{WT}, detected as a germline variant in a patient with Langerhans cell histiocytosis (Satoh, et al. 20112)

Table 2. Non-V600E BRAF mutations detected in SBA and clinical relevance.

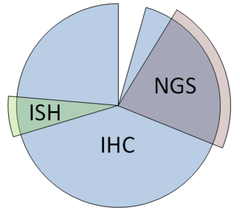

Figure 6. Therapeutically actionable biomarker changes according to platform in SBA. Analysis included specimens whose profiling included NGS (n=55). Fifty-three patients (96%) carried actionable alterations identified by IHC, including three patients who also had alterations identified by ISH, and nineteen patients who also had alterations identified by NGS.

Conclusions

SBA are often treated by the colon cancer protocols. By multi-platform profiling of a large series of SBA, we determined:

- Frequency of KRAS and TP53 mutations in SBA are comparable to CRC; however APC/β-catenin mutations appear at lower frequencies
- 56% of SBA patients do not carry biomarkers of cetuximab resistance such as KRAS, NRAS, PIK3CA (1/31 exhibited mutation) and BRAF V600E.
- We identified novel biomarkers associated with drug sensitivity: MET overexpression (MET inhibitors), low MGMT (alkylating agents -temozolomide), low RRM1 (gemcitabine), anti-HER2 (HER2 amplification), TOP2 (anthracyclines) and taxanes.
- Tumor DNA sequencing identified somatic mutations: BRAF, GNA11, GNAS- MEK inhibitors; ATM- platinum; FBXW7- mTORi; PIK3CA- PI3Ki.
- BRAF V600E mutations are not observed in SBA, however non-V600E mutations were observed in both KRAS WT and MT SBA. The oncogenic role of non-V600E mutations which are prevalent in other cancer types (NSCLC) is being studied, including their therapeutic relevance.
- 59% did not carry ISH or sequence alterations, but had potentially favorable protein biomarkers by the IHC.
- SBA exhibits biologically similar tumorigenesis as colorectal adenocarcinoma, therefore similar treatment guidelines and biomarker testing strategies should be considered.

References

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2. Santini, D., G. Tonini, et al. (2010). "Cetuximab in small bowel adenocarcinoma: a new friend?" Br J Cancer 103: 1305.