Response to angiotensin blockade with irbesartan in a patient with metastatic colorectal cancer

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Received 30 November 2015; revised 3 February 2016; accepted 8 February 2016

Background: A patient suffering from metastatic colorectal cancer, treatment-related toxicity and resistance to standard chemotherapy and radiation was assessed as part of a personalized oncogenomics initiative to derive potential alternative therapeutic strategies.

Patients and methods: Whole-genome and transcriptome sequencing was used to interrogate a metastatic tumor refractory to standard treatments of a patient with mismatch repair-defective metastatic colorectal cancer.

Results: Integrative genomic analysis indicated overexpression of the AP-1 transcriptional complex suggesting experimental therapeutic rationales, including blockade of the renin-angiotensin system. This led to the repurposing of the angiotensin II receptor antagonist, irbesartan, as an anticancer therapy, resulting in the patient experiencing a dramatic and durable response.

Conclusions: This case highlights the utility of comprehensive integrative genomic profiling and bioinformatics analysis to provide hypothetical rationales for personalized treatment options.

Key words: personalized medicine, AP-1 complex, irbesartan, chemo-refractory colon cancer, RNA expression analysis, mismatch repair defective

introduction

Cancers are diseases featuring genetic instability that results in genomically and behaviorally heterogeneous subgroups within each malignancy. This heterogeneity leads to variable responses to therapies among individuals with cancers of the same tumor subtype. The availability of genomic data has greatly enhanced our knowledge of tumor heterogeneity and has defined key driver pathways. These pathways in turn may provide insight into therapeutic rationales for individual malignancies, thereby increasing the potential for superior outcomes and a broader spectrum of drugs to consider for a given malignancy. Here, we present the case of a patient suffering from metastatic colorectal cancer, treatment-related toxicity and resistance to standard chemotherapy and radiation. Biopsies of this metastatic disease were characterized using a whole-genome analysis strategy. This strategy included integrative genomic analysis of whole-genome tumor DNA sequencing and whole transcriptome sequence of tumor RNA. Our analysis revealed a minimally rearranged genome with a high mutational burden due to defective mismatch repair. Analysis of gene expression outliers identified high expression of FOS and JUN, components of the activating protein-1 (AP-1) complex, indicating its potential somatic activation. Based on this analysis, the possibility of treatment targeting the upstream activators of the AP-1 complex was raised and a trial of irbesartan was initiated. The patient’s disease exhibited a profound and durable response to this treatment, which was selected based on genomic and outlier expression analyses.

methods

overview

The study was approved by the University of British Columbia Research Ethics Committee (REB# H12-00137) and written informed consent was obtained from each patient before genomic profiling. Patient identity was anonymized within the research team and an identification code was assigned to the case for communicating clinically relevant information to physicians. The patients consented to potential publication of findings. Raw sequence data and downstream analytics were maintained within a secure computing environment.
histologic studies
Formalin-fixed paraffin-embedded tumor sections underwent immunohistochemical analysis for expression of the MMR proteins MSH2, MSH6, PMS2 and MLH1 and the V600E mutant BRAF protein using standard protocols. For details, see the Methods section in supplementary Appendix S1, available at Annals of Oncology online.

whole-genome and transcriptome sequencing
Whole-genome sequencing was carried out on the pretreatment tumor and blood, and whole-genome sequencing and whole transcriptome sequencing on the metastatic tumor resected from the L3 spinous region. The mean redundant depth of coverage for the pretreatment and metastatic tumors was 50- and 86-fold, respectively. Somatic point mutations, small insertions or deletions (indels) and copy-number alterations, detected in the tumor DNA but not in the germline, were identified (supplementary Table S2, available at Annals of Oncology online). De novo assembly of genomic and transcriptomic data was carried out to detect rearrangements. Publicly available transcriptome sequencing data from normal colon tissue and colon adenocarcinoma were used to explore the expression profile of human genes and transcripts. A within-sample expression rank was also calculated to further infer significance to outlier gene expression levels. For details, see the Methods section in supplementary Appendix S1, available at Annals of Oncology online.

sequencing data availability
Genomic and transcriptomic datasets have been deposited at the European Genome–phenome Archive (EGA, http://www.ebi.ac.uk/ega/) under accession number EGAD00001001876.

results

case report
The patient was a previously healthy 67-year-old female when she presented in May 2010 with moderately differentiated adenocarcinoma of the ascending colon. Right hemicolectomy showed a stage III (pT3N1) adenocarcinoma. She did not tolerate adjuvant capecitabine and oxaliplatin treatment due to significant neutropenia, necessitating dose reduction and G-CSF support. In addition, her course of adjuvant chemotherapy was attenuated to four of eight planned cycles due to severe neuropathy.

After completion of this adjuvant treatment, she proceeded with her active surveillance strategy as per standard guidelines with serum carcinoembryonic antigen (CEA) and CT scan monitoring. In November 2012, she developed a recurrence near the right psoas muscle. This was excised in December with the pathology demonstrating moderately differentiated colonic adenocarcinoma (Figure 1A). Six lymph nodes were negative for disease, but the retroperitoneal resection margin was positive. Immunohistochemical workup showed an abnormal mismatch repair profile with loss of MLH1 protein, with no BRAF V600E mutation identified (Figure 1A).

She completed 45 Gy in 25 fractions of radiotherapy concurrent with capecitabine at 825 mg/m² bid. Again, significant neutropenia led to capecitabine dose reduction.

She then relapsed with disease in the L3 spinous process in October 2013 and received 42 Gy of stereotactic radiotherapy in 10 fractions. In June 2014, the tumor recurred at the same site.

Figure 1. Pathology and positron emission tomography—computed tomography (PET/CT) scans. Hematoxylin and eosin staining (A, left) shows moderately differentiated colonic adenocarcinoma; immunohistochemistry for MLH1 shows loss of staining in tumor cell nuclei with retained staining in background inflammatory cells (centre); c-JUN immunohistochemistry shows strong expression in the tumor cells, note the normal colonic epithelium features staining of the crypt bases only (right). Pretreatment PET/CT scans (B–E) demonstrate fluoroexyglucose (FDG) uptake in the L3 spinous process (B) and in multiple lymph node areas including left supraclavicular, left mediastinal, retrocrural, retroperitoneal, para-aortic and bilateral iliac regions (C). Five weeks after treatment initiation with irbesartan (D and E). FDG activity has resolved in the affected areas.
and she underwent palliative resection of the mass. At this point, she consented to undergo genomic analysis of the tumor resected from the L3 spinous process by the Personalized OncoGenomics (POG) initiative at the BC Cancer Agency (supplementary Table S1, available at Annals of Oncology online).

The genomic analysis revealed overexpression of the FOS and JUN genes that encode the AP-1 transcriptional complex. The transcriptional expression rank of FOS and JUN was in the 98th and 100th percentile in relation to the TCGA colon cancer dataset, respectively, and 94th and 99th in a PAN-cancer comparison against multiple TCGA datasets. Immunohistochemical workup confirmed robust expression of c-JUN protein (Figure 1A). This indicated that mitigation of upstream factors leading to activation of this complex might provide a therapeutic advantage. One such pathway, the renin–angiotensin system, signals through the AP-1 complex and has been reported to be active in colorectal cancers [1]. Furthermore, antiproliferative and apoptotic effects of angiotensin blockade have been reported in human colon cancer cells [2, 3]. Supported by these findings, it was suggested that irbesartan could be a potential option due to the blockade of the renin–angiotensin pathway [4, 5].

The patient had a pretreatment baseline PET/CT scan and started irbesartan at a dose of 150 mg daily in December 2014. She had a follow-up PET/CT at 5 weeks and again at 3 months (Figure 1B–E). Before the therapy, her CEA was elevated at 18 (upper limit of normal 5). After 5 weeks of therapy, this value decreased to a CEA of 3.1. Furthermore, there was virtually a complete functional radiological resolution of her disease (Figure 1D and E). These results are maintained at the 10-month point with CEA at 1.4.

tumor genome analysis

Whole-genome sequencing identified 2183 somatic mutations (SNVs and Indels) predicted to affect protein-coding genes (supplementary Table S2, available at Annals of Oncology online) in the metastatic tumor. The large number of genomic alterations was consistent with defective MMR arising from the loss of MLH1 observed using immunohistochemistry. In support of this observation, two inactivating truncating mutations in MLH1 (E199* and F70Afs) were detected. These mutations were also present in the primary tumor. No germline mutations known to contribute to a hypermutation phenotype were detected (data not shown). Furthermore, a large number of common mutations were observed between the pretreatment versus metastatic tumor (1599 somatic point mutations and indels), consistent with MMR deficiency in the primary lesion. Taken together, these data indicate that the hypermutation phenotype was likely an early somatic event in tumorogenesis facilitated by inactivation of MLH1.

Multiple genes encoding components of the AKT–PI3K–mTOR pathway were disrupted, including a previously reported mutation in PIK3CA (V344M, COSM253279). Mutation of this residue is thought to lead to increased PIK3CA activation through disruption of the interaction between PIK3CA and its regulatory subunit PIK3R1 [6]. PIK3R1 itself harbored two potentially damaging mutations (E65Kfs; S460L531del). In addition, a frame-shift mutation in PTEN (L265fs) was observed, potentially leading to further activation of the AKT–PI3K–mTOR pathway.

Other potentially relevant lesions detected included two frame-shift mutations in APC (L598Wfs; T1556fs), inactivation of which is observed in over 50% of hypermutated colorectal cancers [7], a heterozygous mutation of uncertain significance in TP53 (A83V), a dominant negative mutation in the cell cycle regulator FBXW7 (R479Q, COSM1154291) and potentially inactivating homozygous mutations in genes encoding the TGF-β pathway components BMPR2 (N583Tfs) and SMAD2 (G367D).

In a cohort of sequenced colorectal cancers, mutation of APC, TP53, PIK3CA and FBXW7 were found to be associated with aggressive disease [7]. Finally, a potentially inactivating heterozygous mutation in AGTR1 (S338Fs), which encodes the Angiotensin II receptor, was detected.

Genome-wide copy-number analysis revealed no copy-number gains of known clinical or therapeutic relevance (supplementary Figure S1, available at Annals of Oncology online). Notably, FOS and JUN are not amplified in the tumor. However, single copy losses in chromosomes 3 and 10 were detected in areas harboring the entire coding region of PIK3CA and the first 2 exons of PTEN, respectively (Figure 2A), leading to hemizygosity of the respective mutations in these genes, as described above. Structural analysis from de novo genome assembly revealed 53 rearrangements (translocations, inversions, deletions and duplications) none of which are known to contribute to disease initiation or progression, or to be of utility in therapeutic decision-making (supplementary Table S2, available at Annals of Oncology online).

transcriptome analysis

Among the most significantly differentially expressed genes were those encoding the founding members of two proto-oncogene families, FOS and JUN. Together c-FOS and c-JUN comprise the AP-1 transcriptional complex, which is known to be a key regulator of disease initiation and progression in many cancer types, including colorectal cancer [8, 9]. Expression levels of FOS and JUN were 10- and 4-fold greater than for normal colon tissue, and 3.7- and 4-fold when compared with the mean expression of the malignant colon adenocarcinoma cohort, respectively (equivalent to the 98th and 100th percentile of expression, respectively). Both genes were ranked in the 100th percentile for gene expression within the tumor transcriptome (Figure 2B, supplementary Table S2, available at Annals of Oncology online).

discussion

Innovation in genomic technology in the last decade has greatly advanced the progress of personalized/precision medicine. This case highlights the utility of transcriptome and expression data in combination with genomic mutational data to identify potential therapeutic targets for oncology patients. The Personalized OncoGenomic (POG) Program utilizes parallel sequencing technology and transcriptome information providing a comprehensive map of expressed mutations which provides more information than the standard mutation panels that are currently available commercially. Given that cancer is a disease of genetic instability, this case highlights the significant impact on outcomes when utilizing this technology and repurposing of medications.

This case demonstrated outlier expression of FOS and JUN that was suggestive of oncogene addiction and therefore was potential therapeutic axis for a targeted therapy. Irbesartan, an angiotensin receptor blocker, has demonstrated previous JUN inhibition at the transcriptional and protein level [4, 5].
Colorectal carcinogenesis is activated through the AP-1 transcription factors. The two proto-oncogenes, JUN and FOS, are part of this pathway and have previously been demonstrated to be dysregulated in colorectal carcinomas when compared with normal colon mucosa [10–12]. Pre-clinical models using inhibitors of this pathway have demonstrated some efficacy [13, 14]. In addition to the high expression of FOS and JUN, it is also noted that both the primary and metastatic tumors harbored a heterozygous loss of function allele in the angiotensin receptor type 1 (AGTR1). Although not functionally characterized in the tumor, it is intriguing to postulate that haploinsufficiency of AGTR1 might have further sensitized the pathway to blockade, contributing to the dramatic clinical response. Targeting this pathway was a rational approach, given the heterozygous loss of function of AGTR1 in combination with the dramatic overexpression of the API complex in this patient. It should be noted that AKT pathway inhibitors were also considered as a therapeutic rationale in this case due to potential activation of the PI3K-AKT-mTOR pathway in the tumor due to the observed mutations in PIK3CA, PIK3R1 and PTEN.

The somatic mutations in MLH1 explain the significant number (∼2100) of somatic protein-coding alterations seen in the genome. Hypermutated tumors have been shown to respond to immunotherapy, and the rapid response to the intervention reported herein is reminiscent of the early serum tumor marker responses (between day 14 and day 28) that were seen in mismatch repair-deficient tumors treated with PD1 blockade and predictive of both progression-free and overall survival [15]. The role of immune modulation is intriguing based on the critical roles of AP-1 in leukocyte activation and differentiation in the immune system [16]. Irbesartan has been shown to inhibit AP-1 DNA binding and transcriptional activity in a dose-dependent manner [4]. Irbesartan inhibited production of cytokines by activated T cells and inhibited activation of c-Jun NH2-terminal protein kinase. Further, ARBs have demonstrated preservation of T-cell and monocyte function in patients with multiple organ failure [17]. Together, these data are compatible with the notion that Irbesartan may modulate T-cell function via the AP-1 pathway and it is possible that the patient’s dramatic response was related to immune effects that [18, 19].

**Figure 2.** CNV and expression analysis. CNV plots of chromosomes 3 and 10 in the colonic adenocarcinoma (A); single copy loss in a region containing PIK3CA (left); single copy loss of approximately 20 kb deletes exons 1 and 2 of PTEN (right and inset). Density plots showing relative gene expression levels compared with colon adenocarcinoma (B) for FOS (left) and JUN (right). Green curve indicates expression range in tumor tissue, blue curve depicts expression range in normal tissue, yellow line shows relative expression level in patient tumor tissue, and pink line indicates relative expression level in normal compendium.
contributed to the therapeutic benefit seen in this mismatch repair-deficient tumor.

The TGF-β superfamily is mutated in MSI-H tumors, specifically TGFBR2 and ACVR2A, which may be important for the development of colorectal cancer [20]. Negative feedback may cause a paradoxical increase in TGFβI expression, AP-1 has been shown to act on the TGFβ1 promoter [21]. In our patient, transcriptomic analysis indicated that TGFβ1 expression was high, being in the 83rd percentile among colorectal adenocarcinomas (TCGA COAD), and was elevated relative to normal colon. Attenuation of non-canonical TGF-β signaling has been proposed as a mechanism by which angiotensin II blockade prevents and stabilizes arterial aneurysms in patients with germ-line aberrations in the TGF-β pathway, causing hereditary connective tissue and aneurysmal disease [22]. Furthermore, it has established roles in regulation of immune response that have made it an attractive anticancer target for drug development [18, 19]. Circulating TGFβI concentrations are elevated in Marfan syndrome and decrease after administration of another ARB, losartan, and also β-blocker therapies [23, 24]. The effect of angiotensin II inhibitors on the TGF-β pathway may also have contributed to the activity of irbesartan in this case, directly on the tumor and/or in relation to the immune cell response.

Repurposing drugs in oncology provides another therapeutic strategy [25]. There has been some limited success such as the use of thalidomide in myeloma [26], aspirin in colorectal cancer or metformin in breast cancer [27]. This case highlights the use of whole-genome analysis, including transcriptome data, to identify new candidate therapeutic targets. However, many genomics platforms offer mutation analysis but lack expression information. With comprehensive integrative profiling and bioinformatics analysis, therapy can be made more precise and can provide excellent, and perhaps unexpected, results. As genomic technology linked to clinical implementation advances, further therapeutic options with the possibility of repurposing medications will be realized for more oncology patients.

acknowledgements

We would like to thank the patients and families who participated in this study. We also thank and acknowledge all of the researchers and clinicians who are not listed as authors but worked to make this study possible, particularly Dr Charles Fisher (MD, MHSC, FRCSC) of the Vancouver Spine Surgery Institute, Vancouver General Hospital (VGH), for facilitating surgical tumor tissue extraction and Christine Chow (GPEC) for technical work on the histologic studies. The results shown here are in part based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/.

funding

This research was generously funded through unrestricted philanthropic donations received through the BC Cancer Foundation. MAM acknowledges infrastructure investments from the Canada Foundation for Innovation and the University of British Columbia and the support of the Canada Research Chairs program. No grant numbers applied.

disclosure

The authors have declared no conflicts of interest.

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Observation versus late reintroduction of letrozole as adjuvant endocrine therapy for hormone receptor-positive breast cancer (ANZ0501 LATER): an open-label randomised, controlled trial†

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Received 29 November 2015; revised 26 January 2016; accepted 29 January 2016

Background: Despite the effectiveness of adjuvant endocrine therapy in preventing breast cancer recurrence, breast cancer events continue at a high rate for at least 10 years after completion of therapy.

Patients and methods: This randomised open label phase III trial recruited postmenopausal women from 29 Australian and New Zealand sites, with hormone receptor-positive early breast cancer, who had completed ≥4 years of endocrine therapy [aromatase inhibitor (AI), tamoxifen, ovarian suppression, or sequential combination] ≥1 year prior, to oral letrozole 2.5 mg daily for 5 years, or observation. Treatment allocation was by central computerised randomisation, stratified by institution, axillary node status and prior endocrine therapy. The primary outcome was invasive breast cancer events (new invasive primary, local, regional or distant recurrence, or contralateral breast cancer), analysed by intention to treat. The secondary outcomes were disease-free survival (DFS), overall survival, and safety.

Results: Between 16 May 2007 and 14 March 2012, 181 patients were randomised to letrozole and 179 to observation (median age 64.3 years). Endocrine therapy was completed at a median of 2.6 years before randomisation, and 47.5% had tumours of >2 cm and/or node positive. At 3.9 years median follow-up (interquartile range 3.1–4.8), 2 patients assigned letrozole (1.1%) and 17 patients assigned observation (9.5%) had experienced an invasive breast cancer event (difference 8.4%, 95% confidence interval 3.8% to 13.0%, log-rank test $P = 0.0004$). Twenty-four

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†Prior presentations: This work describes original data. It has been presented in part at the American Society of Clinical Oncology Annual Meeting in Chicago, IL, in May 2015. The authors assume full responsibility for analyses and interpretation of these data.