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Editorial

Cancer Prevention Research

Molecular Predicators of Duodenal Familial Adenomatous Polyposis Chemoprevention: Do Chemopreventive Drugs Hit Their Presumed Molecular Targets?

Imad Shureiqi

Abstract

Patients with familial adenomatous polyposis (FAP) have an increased risk of developing duodenal adenomas and adenocarcinomas. In previous trials, sulindac (a cyclooxygenase inhibitor) alone failed to significantly suppress duodenal tumorigenesis in FAP patients, but sulindac plus the tyrosine kinase inhibitor erlotinib significantly reduced duodenal polyp burden. Delker and colleagues report in this issue (beginning on page 4) on transcriptome analyses that aimed to identify the molecular targets mediating the response to sulindac–erlotinib. Their exploratory transcriptome analyses suggested that sulindac–erlotinib suppressed duodenal polyposis via inhibiting Wnt/β-catenin, EGFR, and cyclooxygenase pathways. This perspective discusses the significance and limitations of the study. *Cancer Prev Res; 11(1); 1–3.* ©*2017 AACR.*

See related article by Delker et al., p. 4

Patients with familial adenomatous polyposis (FAP) have an increased lifetime risk of developing duodenal adenomas that can progress to life-threatening duodenal adenocarcinomas (1). NSAIDs, especially sulindac, have demonstrated clear chemopreventive effects, inhibiting the development of colonic adenomas in FAP patients (2–6). However, sulindac's chemopreventive effects on FAP-related duodenal adenomas were nonsignificant in one study (5) and significant only for diminutive polyps (2 mm or smaller) in another study (6). Unfortunately, the risk of tumorigenesis progression is higher for larger polyps. Celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, significantly suppressed FAP duodenal polyposis at a dose of 400 mg twice daily (7). However, this celecoxib dose was later found to increase cardiovascular risk (8). Therefore, long-term endoscopic surveillance of the upper gastrointestinal tract and removal of duodenal polyps remains the standard of care for FAP patients. Effective chemopreventive interventions to prevent duodenal tumors in FAP patients are much needed.

Preclinical models have demonstrated that a convergence of Wnt/β-catenin and EGFR signaling drives intestinal tumorigenesis (9), which is further potentiated by COX facilitation of prostaglandin E2 (PGE2) synthesis (10). These and other preclinical data formed the rationale for a randomized clinical trial of the combination of sulindac and erlotinib (an inhibitor of an EGFR-related tyrosine kinase) as chemoprevention for duodenal polyps in FAP patients (11). This landmark study showed that sulindac–erlotinib treatment significantly reduced duodenal polyp burden and count versus placebo in FAP patients (11). Furthermore, the combination treatment suppressed the duodenal polyp burden most effectively among patients with a higher baseline duodenal polyp burden, unlike single-agent sulindac, which suppressed only diminutive duodenal polyps in a prior study (6). Moreover, the combination therapy produced mainly grade 1–2 toxicities, suggesting that it is a feasible approach to chemoprevention for FAP-related duodenal polyposis. Taken together, these findings suggested that the combination of sulindac and erlotinib was more effective at preventing FAP-related duodenal polyposis than was single-agent sulindac, although a head-to-head comparison would be needed before drawing definitive conclusions.

The chemopreventive effects of sulindac and erlotinib were however only partial, with at least 25% of patients having either unchanged or increased polyp burden. Therefore, identification of the critical molecular mechanisms that mediate the response to sulindac–erlotinib combination therapy could identify biomarkers that can predict clinical benefit and detect molecular targets for developing better chemopreventive agents.

In the current issue of this journal, the article by Delker and colleagues (12) reports the results of transcriptome analyses of duodenal samples from patients in the previously published trial (8). Normal-appearing mucosa and polyp samples from 10 patients who received sulindac–erlotinib combination therapy and 10 patients who received placebo were used for profiling of the molecular targets that mediated the response to combination therapy. Endoscopic duodenal biopsy samples were obtained from normal-appearing mucosa at the beginning and end of the 6-month treatment period. Polyp samples, however, were obtained only at the end of treatment. Although the authors did not explain how they selected treatment cohorts' subgroups for molecular profiling, it appears that the included samples were from patients who had the most pronounced responses in the combination therapy group and the most disease progression in

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the placebo group. This inference is based on a comparison of the data in Table 1 of the current article (12) and Table 2 in the previously published article (11). The selection of the patients for molecular profiling clearly enhanced the contrast in clinical responses between the treatment and placebo groups; the median change in the sum of the diameters of the polyps from pre- to posttreatment was markedly larger for the molecularly profiled subgroups than for the original trial cohort (profiled subgroup vs. original cohort: −63.5 mm versus −37.9 mm for the combination therapy group and 91.5 mm vs. 30.6 mm for the placebo group). Therefore, these relatively small subgroups (27% of each original cohort), which were selected for molecular profiling, have more dramatic clinical outcome changes than the overall cohorts.

The current article reports results of RNA sequencing of 17 baseline (pretreatment) normal tissue samples and 32 posttreatment polyps and 20 normal tissue samples. To improve sequencing depth and reduce amplification biases, 6 posttreatment polyp samples from each group (treatment and placebo) were selected for further gene expression analyses using a panel of 475 human genes involved in inflammation and immunity. The mRNA expression findings of *MMP7*, *CD44*, *FOS*, *TM4SF5*, *EGFR*, and *PTGS2* were confirmed using a qRT-PCR. Natural killer (NK) cells were counted using immunohistochemistry (IHC) analyses of CD56 expression in polyp samples from all 20 included patients. In both groups, 3 colonic polyp samples were stained for CD56 in addition to the duodenal polyp samples (19 for placebo and 7 for sulindac and erlotinib treatment subgroups).

Extensive exploratory analyses using low cut-off points (fold changes of 1.5–2.0) and adjustment for various small subgroups identified a pattern of differentially expressed genes that separated sulindac–erlotinib–treated polyps from placebo-treated polyps. Comparative transcriptome analyses of posttreatment polyps with pretreatment normal mucosa samples showed 977 differentially expressed genes in the placebo group versus 51 in the sulindac–erlotinib–treated group. Authors appropriately interpreted this difference as suggestive of sulindac–erlotinib treatment reversing tumorigenesis-induced gene expression changes. The top 5 pathways identified by Ingenuity upstream regulator analyses of 2,637 differentially expressed genes (1.5-fold change and FDR <0.05 cut-off points) were TNF, β-catenin, EGF, EGFR, and PGE2. In the inflammation and immunity-focused transcriptome analyses, PGE2 was one of the top 5 pathways identified. These differential expression patterns suggested that the combination treatment modulated the Wnt/β-catenin, PGE2, and EGFR signaling pathways. When posttreatment polyps were compared with posttreatment normal mucosa, 493 genes were found to be differentially expressed in the placebo group and only one gene in the sulindac–erlotinib treatment group. In contrast, a comparison of posttreatment normal mucosa in the combination therapy and placebo groups detected no differentially expressed genes.

These findings suggest that (i) the sulindac–erlotinib treatment had no impact on mRNA expression in normal mucosa; (ii) gene expression patterns differed significantly, as expected, between normal mucosa and tumor tissue; and (iii) this differential expression pattern could be modulated by sulindac–erlotinib treatment especially in the Wnt/β-catenin, EGFR, and PGE2 pathways. The observation that Wnt/β-catenin, EGFR, and PGE2 pathways were modulated by the sulindac–erlotinib treatment is very interesting because it could provide some evidence that sulindac and erlotinib modulate their presumed targets to exert their chemopreventive effects. However, the reported transcriptome analyses are exploratory and require validation by independent assays, preferably in separate groups of patient samples to ensure that the observed differences are not secondary to false discoveries. Unfortunately, quantitative RT-PCR validation of the differentially expressed targets in each of these 3 pathways showed significant differential expression for only *FOS* mRNA (in the EGFR pathway); the mRNA expression levels for the other tested downstream genes (*CD44*, *MMP7*, *TM4SF5*, *EGFR*, and *PTGS2*) were not significantly different between combination-treated and placebo-treated polyps. These findings leave the transcriptome analysis results without confirmation that Wnt/β-catenin and PGE2 were in fact modulated by sulindac–erlotinib to suppress duodenal polyposis. An additional important concern is that this study did not examine the biological significance of the identified mRNA alterations for either protein expression or enzymatic activity. Addressing this issue is important because the biological relevance of tumor mRNA alterations could be modified by downstream translational and posttranslational changes.

The findings from the Ingenuity Pathway Analyses of the transcriptome data also suggested that activation of the NK-cell immune response may be a mechanism for the chemopreventive activity of the combination therapy. In IHC analyses, NK-cell count was 1.43-fold higher in the combination-treated polyps than in the placebo-treated ones. This difference was, however, statistically nonsignificant (95% CI, 0.77–2.66; 0.2641). Thus, although these findings intriguingly suggest that sulindac–erlotinib therapy might activate an antitumor immune response, they remain preliminary and require confirmation.

Several other important issues are also left unanswered by the current study. One concern is that the combination treatment only partially induced polyp regression, and the differential gene expression was identified by comparing sulindac–erlotinib–treated polyps that persisted despite 6 months of combination treatment with placebo-treated polyps. This raises the question of whether these residual polyps had developed resistance mechanisms that allowed them to persist despite Wnt/β-catenin, EFGR, and PGE2 pathway suppression or whether the inhibition of these pathways induced by sulindac and erlotinib was inadequate to induce complete polyp regression. Another related question is whether combination therapy with sulindac and erlotinib modulated the Wnt/β-catenin, EGFR, and PGE2 pathways in patients whose duodenal polyps failed to respond, a subgroup of at least 25% of the original cohort. Unfortunately, these patients were not included in the currently reported analyses; thus, the mechanisms underlying treatment resistance remain unknown. Identifying the molecular changes that predict resistance to sulindac–erlotinib treatment could open an opportunity to develop chemopreventive agents that can effectively target these mechanisms. It could also help select patients whose polyps are likely respond to sulindac–erlotinib chemoprevention and thus spare nonresponders treatment side effects.

The long quest to validate molecular target modulation by chemopreventive agents in human studies has been challenging because of the heterogeneity of tumors and individual patients. Researchers encounter difficulties in procuring and analyzing human samples, which are commonly limited in amount and heterogeneous in composition, especially in regard to the stromal

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and epithelial cell components. Moreover, in the "omics" era, the number of potential targets that might contribute to response or resistance is ever increasing, and the screening study spectrum is vastly expanding. Delker and colleagues' article represents a courageous attempt to rise to these challenges and provides a glimpse of molecular mechanisms that matches the ones predicted in preclinical studies of sulindac and erlotinib. This faint glimpse could signal that we are on the road to identifying the true mechanistic significance of molecular targets for chemopreventive drug response. Nonetheless, the limitations in the answers provided by the current study demonstrate that we still have a long way to go.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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