Interaction of Molecular Markers and Physical Activity on Mortality in Patients with Colon Cancer

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Abstract Purpose: Physical activity in colon cancer survivors has been associated with lower cancer recurrences and improved survival. Whether molecular features of the tumor portend more or less likelihood for benefit from exercise is unknown. Experimental Design: Using two large prospective cohort studies with physical activity assessments after colon cancer diagnosis, we examined expression of fatty acid synthase, p53, p21, and p27 and mutational status of K-ras and phosphatidylinositol 3-kinase (PI3KCA). We calculated hazard ratios (HR) of colon cancer–specific mortality, adjusted for tumor and patient characteristics, and tested for molecular interactions with exercise. Results: In a cohort of 484 men and women with stage I to III colon cancer, patients who engaged in at least 18 metabolic equivalent task (MET)–hours per week after diagnosis had an adjusted HR for colon cancer–specific mortality of 0.64 [95% confidence interval (95% CI), 0.33–1.23] and for overall mortality of 0.60 (95% CI, 0.41–0.86). A statistically significant interaction was detected based on p27 expression (P = 0.03). For tumors with loss of p27 (n = 195), physical activity of ≥18 MET-hours/week led to a HR for colon cancer mortality of 1.40 (95% CI, 0.41–4.72), compared with those with <18 MET-hours/week. However, for tumors with expression of p27 (n = 251), the adjusted HR was 0.33 (95% CI, 0.12–0.85). Molecular status of fatty acid synthase, K-ras, p53, p21, and PI3KCA did not influence the association between exercise and colon cancer–specific or overall mortality. Conclusion: The benefit of physical activity on outcomes in patients with stage I to III colon cancer may be influenced by p27 status. Further studies are warranted to confirm these findings. (Clin Cancer Res 2009;15(18):OF1–6)

Physically active people have a reduced risk of developing colon cancer (1–11). A meta-analysis of 19 cohort studies showed a statistically significant 22% reduction in the risk of colon cancer in active males and 29% reduction in active females (12). The IARC concluded that the evidence supports a causal relation between inactivity and colon cancer risk (13). In two large prospective observational studies of colon cancer patients, physical activity after colon cancer diagnosis was associated with significant improvements in colon cancer recurrences (14) or colon cancer–specific mortality (15) and overall mortality (14, 15). Colon cancer survivors who engaged in higher levels of physical activity experienced a 50% to 60% improvement in long-term outcomes compared with inactive patients.

Studies have suggested that energy balance and physical activity influence certain molecular features of tumors. Obesity and/or reduced physical activity have been associated with colon cancers with p53 overexpression (16) and K-ras mutations (17, 18). Fatty acid synthase (FASN) is physiologically regulated by energy balance, and high-carbohydrate/low-fat diets up-regulate FASN (19). On the contrary, exercise and energy restriction down-regulate FASN through AMP-activated kinase (20).

Obesity, insulin, and insulin-like growth factor I influence growth and inhibit apoptosis through phosphatidylinositol

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Colorectal cancer is the fourth most common cancer diagnosed in the United States. Eighty percent of patients are diagnosed with nonmetastatic disease and treated with curative intent with surgery with or without adjuvant therapy. Despite standard therapy, up to 40% of patients will recur and adjunctive therapy to standard treatment is of great interest. Recent data suggest that colon cancer survivors who are physically active have improved disease-free and overall survival after diagnosis compared with those that are relatively inactive. To further explore those observations, we tested whether physical activity is more or less beneficial toward survival in colon cancer subgroups as defined by molecular markers.

Translational Relevance

Materials and Methods

Study population. We used the databases of two large prospective cohort studies, the Nurses’ Health Study (n = 121,700 women followed since 1976), and the Health Professional Follow-up Study (n = 51,500 men followed since 1986). Every 2 y, participants have been sent follow-up questionnaires to update information on potential risk factors and to identify newly diagnosed cancer and other diseases. This study was approved by the Human Subjects Committees at Brigham and Women’s Hospital and the Harvard School of Public Health, both in Boston, Massachusetts.

Measurement of colon cancer and mortality. On each biennial follow-up questionnaire, participants were asked whether they had a diagnosis of colon cancer during the previous 2 y. When a participant (or next of kin for decedents) reported colon cancer, we sought permission to obtain medical records. Study physicians, while blinded to exposure data, reviewed all records related to colon cancer, and recorded American Joint Committee on Cancer tumor stage and tumor location. For nonresponders, we searched the National Death Index to discover deaths and ascertain any diagnosis of colon cancer that contributed to death or was a secondary diagnosis. The ascertainment of cases of colon cancer has been described in detail (29). The response rate for participants who had nonfatal outcomes was 96% of the possible number of person-years. We collected paraffin-embedded tissue blocks from hospitals where colon cancer patients underwent resections of primary tumors. Tissue sections from all colon cancer cases were reviewed by a pathologist (S.O.). Tumor grade was categorized as high (≤50% glandular area) or low (>50% glandular area). Based on availability of tissue samples, the current analyses have up to 487 tumor samples with physical activity assessments.

Patients were observed until death or June 2006, whichever came first. Ascertainment of deaths included reporting by the family or postal authorities. In addition, the names of persistent nonresponders were searched in the National Death Index (30). The cause of death was assigned by physicians blinded to other clinical and lifestyle information. In rare patients who died as a result of colon cancer not previously reported, we obtained medical records with permission from next of kin. More than 98% of deaths in the cohorts were identified by these methods (31, 32).

Exposure assessment. Since 1986, leisure-time physical activity has been assessed every 2 y in both cohorts, as previously described and validated against subject diaries (33, 34). Subjects reported duration of participation (ranging from 0-11 or more hours per week) on walking (along with usual pace), jogging, running, bicycling, swimming laps, racket sports, other aerobic exercises, lower intensity exercise (yoga, toning, stretching), or other vigorous activities.

We have previously reported that women with colon cancer who were more physically active had a statistically significant improvement in colon cancer–specific mortality compared with those engaging in minimal leisure-time physical activity (15). In that analysis, as well as the present one, to avoid bias due to declining activity, physical activity was not updated (only a single postdiagnosis measurement was used).

Each activity on the questionnaire was assigned a metabolic equivalent task (MET) score (35). One MET is the energy expenditure for sitting quietly. MET scores are defined as the ratio of the metabolic rate associated with specific activities divided by the resting metabolic rate. The values from the individual activities were summed for a total MET-hours per week score. Based on prior studies of physical activity in colon cancer survivors, patients who engaged in at least 18 MET-hours per week had significantly improved colon cancer–specific mortality (14, 15). For primary analyses, we dichotomized physical activity to <18 MET-hours per week and ≥18 MET-hours per week.

Since our prior studies have found an association between physical activity after diagnosis and survival (14, 15), the first physical activity assessment collected at least 1 y but no >4 y after diagnosis (median, 17 mo) was used to avoid assessment during the period of active treatment.

Covariates. Stage of disease, grade of tumor differentiation, year of diagnosis, and location of tumor were extracted from the medical record. The time interval between cancer diagnosis and assessment of activity was also adjusted for in these analyses. Body mass index (BMI) was also obtained from the biennial questionnaire at the time of the respective physical activity assessment.

Immunohistochemistry for FASN, p53, p21, and p27. Tissue microarrays (TMA) were constructed and immunohistochemistry for FASN, p53, p21, and p27 was done as previously described (36–39). Appropriate positive and negative controls were included in each run for each marker’s immunohistochemistry. All immunohistochemically stained slides were interpreted by a pathologist (S.O.) blinded from any other laboratory data. FASN expression was categorized as negative (0 or weak expression) or positive (strong expression). For p53, we visually estimated the fraction of tumor cells with strong and unequivocal nuclear staining, by examining at least two tissue cores in TMA, or the whole tissue section in each case for which there was not enough tissue for TMA or results were equivocal in TMA. p53 positivity was defined as 50% or more of tumor cells with moderate or strong staining. For p21 immunohistochemistry, normal colonic mucosa or rare mesenchymal cells served as internal positive control. We visually estimated the fraction of tumor cells expressing p21, using the whole tissue section on a single slide for every case. p21 expression was interpreted as loss in ≥20% of cells were positive and “expressed” if ≥20% of cells were positive. The extent of

3-kinase (PIK3CA), which in turn activates Akt/protein kinase B (PKB) via phosphorylation (21). Phosphorylation of Akt (phospho-Akt) results in cell proliferation and escape from apoptosis (22). Approximately 45% of colon cancers overexpress phospho-Akt (23), which inhibits transcription and promotes degradation of the cyclin-dependent kinase inhibitor p27 (24), and in vitro studies show that insulin and insulin-like growth factor I similarly result in down-regulation of p27 (25). In animals, p27 expression increased in a dose-dependent manner in response to energy restriction and/or physical activity (26–28).

Given the association between postdiagnosis physical activity and improved survival outcomes in patients with surgically resected colon cancer (14, 15) and the influence of physical activity on molecular features of tumors, we sought to determine whether the influence of physical activity on patient survival differs depending on molecular features of the tumor. Using two large, prospective cohorts with self-reported postdiagnosis physical activity data and tumor blocks, we tested the interaction between exercise and molecular markers on colon cancer–specific and overall mortality in patients with stage I, II, and III colon cancer.

Clinical Therapy

OF2 www.aacrjournals.org

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nuclear p27 expression was visually estimated using whole tissue sections, and interpreted as “loss” (no staining, only weakly staining, or <20% of tumor cells positive for moderate/strong staining) or expressed if moderate/strong positive in ≥20% of cells.

A random selection of 114 to 246 cases was reexamined for each marker by a second pathologist (p53 and FASN by K.N.; p21 and p27 by K.S.) unaware of other data, and concordance rates and κ coefficients between the two pathologists were as follows: 0.87 (κ = 0.75; n = 118) for p53, 0.93 (κ = 0.57; n = 246) for FASN, 0.83 (κ = 0.62; n = 179) for p21, and 0.94 (κ = 0.60; n = 114) for p27.

Pyrosequencing for K-ras and PIK3CA. Genomic DNA was extracted from dissected tumor tissue sections using QIAmp DNA Mini kit (Qiagen; ref. 40). Normal DNA was obtained from colonic tissue at resection margins. Whole genome amplification of genomic DNA was done by PCR using random 15-mer primers. PCR and Pyrosequencing were done as previously described (40, 41).

Statistical analysis. Cox proportional hazards models were used to calculate hazard ratios of colon cancer-specific death from colon cancer, adjusted for other risk factors for cancer survival. Death from colon cancer was the primary end point and deaths from other causes were censored. Participants were followed from the date of return of postdiagnosis physical activity assessment to either death or June 2006, whichever came first. Modeling was done by entering physical activity in the model and stratifying by the molecular marker and by entering the molecular marker and stratifying by physical activity. Tests of interactions between physical activity categories and molecular markers were assessed by entering in the model the cross product of the dichotomized physical activity variable and the dichotomized molecular marker. No formal adjustments for multiple hypothesis testing were done but considered when interpreting results. All analyses used SAS version 8.0 (SAS Institute, Inc.).

Results

Baseline characteristics. At the time of analyses of these two cohorts, 1,024 subjects had available tumor blocks. Of those, 678 were colon cancers. Eight patients were excluded due to having another cancer diagnosis within 3 years of the colon cancer, 25 patients were excluded for not having a diagnosis of colon in the time frame of 1986 to 2006 (physical activity assessments began in 1986), and 81 patients were excluded for stage IV colon cancer (eligible sample size based on blocks was 564). Of those patients, 488 had a measurement of physical activity within 4 years of diagnosis (median time to assessment, 17 months with 95% within 30 months after diagnosis) but 4 patients died within 6 months of the activity assessment and thus were excluded (consistent with our prior analyses). Thus, 484 colon cancer patients without evidence of metastatic disease at diagnosis were included in these analyses (Table 1). When considering all subjects in the cohort that meet the inclusion/exclusion criteria (colon cancer, not stage IV at diagnosis, time frame of study, having appropriate postdiagnosis physical activity assessment), no significant changes were seen in baseline characteristics with and without blocks available for analysis (data not shown). Sixty-three percent (n = 307) reported physical activity levels of <18 MET-hours per week, whereas 37% (n = 177) engaged in 18 or greater MET-hours per week. The median age at diagnosis, median BMI, year of diagnosis distribution, and median time from diagnosis to physical activity assessment were similar between the two exercise categories. Those engaging in <18 MET-hours per week were more likely to be female, whereas those with at least 18 MET-hours per week of exercise were more likely to be male. Stage distribution was fairly similar, although there was a higher percentage of stage I versus II patients in the less active cohort and higher percentage of stage II versus I patients in the more active cohort.

Impact of physical activity on outcomes by molecular markers. We have previously reported that higher levels of physical activity after colon cancer diagnosis was associated with better colon cancer-specific and overall mortality (14, 15). We did subgroup analyses by individual molecular markers comparing <18 MET-hours/week of exercise to at least 18 MET-hours/week on colon cancer–specific mortality (Table 2). A protective association for increased physical activity was detected regardless of FASN, K-ras, p53, or p21 status; no significant interactions were detected for these markers. In contrast, the effect of physical activity on patient outcome seemed to differ significantly according to p27 status (Pinteraction = 0.03). For patients with loss of p27, regular physical activity conferred no benefit, whereas among patients with tumoral expression of p27 intact, physical activity was associated with a significant reduction in colon cancer–specific mortality (hazard ratio, 0.32; 95% confidence interval, 0.12–0.83). The benefit associated with physical activity seemed to be absent among patients with PIK3CA mutations, although a test for statistical interaction was not significant.

Increased levels of physical activity were associated with a statistically significant 40% improvement in overall mortality in this cohort of stage I to III colon cancer patients with tumor blocks and physical activity assessment at least 6 months after diagnosis (Table 3). These results were largely unchanged by status of FASN, K-ras, p53, p21, and PIK3CA in the primary tumors.

We tested whether any of the markers confounded the previously reported associations between physical activity and either colon cancer–specific mortality or overall mortality. Adding the status of these six markers, either individually or all in the same model, did not impact on the multivariate hazard models (data not shown).

Discussion

Prospective observational data suggest that physically active colon cancer survivors have lower rates of cancer recurrence...
and improved survival compared with inactive survivors (14, 15). However, as with any oncological intervention, it is likely that not all patients derive a benefit from exercise. We tested molecular pathways that have been associated with energy balance to determine if a population of colon cancer survivors particularly benefits from physical activity. Surveying a variety of molecular events, we found that the benefit associated with physical activity differed significantly according to p27 expression. Patients with loss of p27 did not seem to benefit from physical activity but those with expression of p27 and who were physically active (at least 18 MET-hours/week) had a 68% improvement in colon cancer–specific mortality compared with those with p27 expression but not physically active.

### Table 2. Subgroup analyses by molecular markers for colon cancer–specific mortality comparing high to low levels of physical activity

<table>
<thead>
<tr>
<th>Events/n</th>
<th>MET-hours/week ≥18 vs MET-hours/week &lt;18; hazard ratio † (95% CI)</th>
<th>P_{interaction}</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients with tumor blocks*</td>
<td>50/484</td>
<td>0.64 (0.33-1.23)</td>
</tr>
<tr>
<td>FASN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>41/390</td>
<td>0.61 (0.30-1.25)</td>
</tr>
<tr>
<td>Positive</td>
<td>4/66</td>
<td>0.95 (0.11-8.06)</td>
</tr>
<tr>
<td>K-ras</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>25/284</td>
<td>0.71 (0.28-1.82)</td>
</tr>
<tr>
<td>Mutation</td>
<td>22/169</td>
<td>0.42 (0.15-1.18)</td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20/276</td>
<td>0.46 (0.16-1.35)</td>
</tr>
<tr>
<td>Positive</td>
<td>26/192</td>
<td>0.64 (0.26-1.59)</td>
</tr>
<tr>
<td>p21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost</td>
<td>37/360</td>
<td>0.87 (0.42-1.81)</td>
</tr>
<tr>
<td>Expression</td>
<td>8/90</td>
<td>0.10 (0.01-0.98)</td>
</tr>
<tr>
<td>p27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost</td>
<td>28/251</td>
<td>0.32 (0.12-0.85)</td>
</tr>
<tr>
<td>Expression</td>
<td>17/195</td>
<td>1.40 (0.41-4.72)</td>
</tr>
<tr>
<td>p16/INK4A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p16/INK4A Wild-type</td>
<td>33/340</td>
<td>0.59 (0.26-1.33)</td>
</tr>
<tr>
<td>p16/INK4A Mutation</td>
<td>7/69</td>
<td>1.25 (0.25-6.40)</td>
</tr>
</tbody>
</table>

*Block cohort represents subjects who had tumor blocks available, postdiagnosis physical activity data and stages I to III at diagnosis. The number of patients varies for each marker because some samples were indeterminant for certain markers.

†Adjusted for age, gender, stage, year of diagnosis, histology grade, BMI, and time of physical activity assessment.

### Table 3. Subgroup analyses by molecular markers for overall mortality comparing high to low levels of physical activity

<table>
<thead>
<tr>
<th>Events/n</th>
<th>MET-hours/week ≥18 vs MET-hours/week &lt;18; hazard ratio † (95% CI)</th>
<th>P_{interaction}</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients with tumor blocks*</td>
<td>152/473</td>
<td>0.60 (0.41-0.86)</td>
</tr>
<tr>
<td>FASN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>128/390</td>
<td>0.65 (0.44-0.96)</td>
</tr>
<tr>
<td>Positive</td>
<td>18/66</td>
<td>0.28 (0.09-0.83)</td>
</tr>
<tr>
<td>K-ras</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>83/284</td>
<td>0.58 (0.34-0.98)</td>
</tr>
<tr>
<td>Mutation</td>
<td>62/169</td>
<td>0.56 (0.31-1.00)</td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>85/276</td>
<td>0.73 (0.45-1.19)</td>
</tr>
<tr>
<td>Positive</td>
<td>65/192</td>
<td>0.44 (0.24-0.80)</td>
</tr>
<tr>
<td>p21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost</td>
<td>112/360</td>
<td>0.65 (0.43-1.00)</td>
</tr>
<tr>
<td>Expression</td>
<td>31/90</td>
<td>0.57 (0.24-1.36)</td>
</tr>
<tr>
<td>p27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lost</td>
<td>57/195</td>
<td>0.67 (0.36-1.24)</td>
</tr>
<tr>
<td>Expression</td>
<td>82/251</td>
<td>0.56 (0.34-0.93)</td>
</tr>
<tr>
<td>p16/INK4A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>106/340</td>
<td>0.55 (0.35-0.86)</td>
</tr>
<tr>
<td>Mutation</td>
<td>23/69</td>
<td>0.80 (0.31-2.03)</td>
</tr>
</tbody>
</table>

*Block cohort represents subjects who had tumor blocks available, post diagnosis physical activity data.

†Adjusted for age, gender, stage, year of diagnosis, histology grade, BMI, and time of physical activity assessment.
Personalized medicine is a growing goal in the treatment of cancer patients (42). It is clear that individual pharmacologic interventions will not impact all patients with the same cancer type. As such, there is growing interest to find markers that better differentiate patients that are likely to benefit from a treatment from patients that have little to no chance of deriving benefit. Similarly, as evidence grows that nondrug therapies can influence patients with established cancer, there is a need to better delineate subpopulations of cancers that may or may not be more likely to be impacted by an intervention. Given the consistent evidence suggesting that physical activity reduces colon cancer recurrences in early stage patients (14, 15, 43), we hypothesized that certain characteristics of a patient's tumor may interact with the biological effects of exercise. With the exception of p27, no such interaction was detected for colon cancer-specific or overall mortality. Further, although the P for interaction between p27 and physical activity was statistically significant for colon cancer-specific mortality (P = 0.03), there was no significant interaction for overall mortality (P = 0.37).

In preclinical models, higher levels of p27 expression were detected in chemically induced malignancies in animals that were energy restricted compared with those not restricted (26–28). Such an effect will arrest cell cycle progression. Thus, the interaction detected in our data is consistent with a hypothesis that energy restriction by physical activity could influence p27-expression tumors by cell cycle arrest inhibiting growth. Excess energy balance may have a much stronger impact on tumor behavior if tumor cells can up-regulate p27 to arrest cell cycle, than if tumor cells have lost the ability to up-regulate p27, possibly through the constitutive activation of the AKT1 pathway. However, it is not clear why no such benefit was detected for overall mortality. One explanation is that those with p27-expressing tumors clearly derive a benefit from exercise related to their colon cancer but that exercise still is beneficial to all patients regardless of p27 status and equally protective for overall mortality related to noncancer-related causes (e.g., cardiovascular disease). Another possibility is that the finding for colon cancer–specific mortality is by chance alone, a risk of multiple hypothesis testing.

The use of the Nurse’s Health Study and Health Professional Follow-up Study cohorts provides multiple advantages to study molecular-environment interactions. Diet and lifestyle are prospectively collected and entered into a database blind to a patient’s diagnosis. Data are updated every 2 years. Tumor block ascertainment has been fairly high (~60%). Subjects are treated at hospitals throughout the United States and represent diverse treatment approaches that could be considered generalizable on a population level. However, a limitation of this study is that cancer treatment data are not available for most patients in our cohorts. Nonetheless, it is unlikely that chemotherapy use differed according to molecular characteristics of the tumor beyond typical pathologic features like stage of disease and grade of differentiation (which are adjusted for in multivariate models). In addition, beyond cause of mortality, data on cancer recurrences were not available in these cohorts. Nonetheless, given the median survival for metastatic colon cancer was approximately 10 to 12 months during much of the time period of this study (44), colon cancer–specific survival should be a reasonable surrogate for cancer-specific outcomes. Finally, these data are limited to patients that were alive to have their physical activity assessed after diagnosis (median, 17 months). As such, conclusions are limited to that population.

In conclusion, this large prospective study of colon cancer patients confirms an association between physical activity and lower colon cancer–specific and overall mortality in colon cancer survivors. However, a molecular signature influencing this association was not clearly detected. Although p27 status may be relevant, these findings require confirmation in independent populations of colon cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank the Nurses’ Health Study and Health Professionals Follow-up Study cohort participants who have generously agreed to provide us with biological specimens and information through responses to questionnaires; hospitals and pathology departments throughout the U.S. for generously providing archival tumor specimens; and Walter Willett, Susan Hankinson, and many other staff members who implemented and have maintained the cohort studies.

References

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Clin Cancer Res  Published OnlineFirst September 1, 2009.

Updated version  Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-0496

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