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Study on the relationship between abnormal imaging manifestations of adipose tissue around the primary tumor of colon cancer, genomics and recurrence probability

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► ***Original article***

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ABSTRACT

Background: This study aims to explore the relationship between abnormal imaging manifestations of adipose tissue around the primary tumor of colon cancer, genomics, and recurrence probability, in order to deepen our understanding of the pathological characteristics and molecular mechanisms of colon cancer. ***Materials and Methods:*** We collected imaging data of a group of colon cancer patients and obtained relevant genetic information through genomic analysis. Statistical methods were used to quantitatively analyze the abnormal imaging manifestations of adipose tissue around the primary tumor of colon cancer. At the same time, the patient's clinical course is followed to assess the probability of recurrence. ***Results:*** The study found that abnormal imaging manifestations of adipose tissue surrounding primary colon cancer tumors were significantly associated with specific gene mutations. Some specific genetic mutations are positively correlated with abnormal signals on imaging, while others are negatively correlated. Furthermore, after long-term follow-up, we observed a relatively higher probability of recurrence in patients with genetic variants associated with imaging abnormalities. ***Conclusion:*** The abnormal imaging manifestations of adipose tissue around the primary tumor of colon cancer can be used as one of the auxiliary indicators for molecular classification of colon cancer. The association between specific genetic variants and imaging abnormalities suggests possible molecular mechanisms and provides new clues for personalized treatment. Furthermore, the association of imaging abnormalities with recurrence probability emphasizes its potential importance in prognostic assessment.

INTRODUCTION

Colon cancer is a leading cause of malignancy worldwide, causing significant concern due to its high prevalence and mortality rates [1, 2]. Despite advancements in early detection, diagnosis, and management of colon cancer in recent years [3, 4], its pathogenesis remains complex and not fully understood [5, 6]. The effectiveness and prognosis of colon cancer treatments are greatly influenced by the molecular characteristics of the tumors, underscoring the importance of investigating the molecular mechanisms underlying colon cancer as a critical research direction [7, 8].

Progress in genomic research has shed light on numerous critical genes and pathways involved in the initiation and progression of colon cancer [9, 10]. For instance, mutations in the adenomatous polyposis coli (APC) gene and the *Wnt* signaling pathway are known to play a significant role in colorectal carcinogenesis [11, 12]. Additionally, studies have identified the *KRAS*, B-Raf proto-oncogene (*BRAF*), and tumor protein p53 (*TP53*) genes as essential factors in the molecular landscape of colon cancer [13, 14]. However, the role of adipose tissue surrounding the primary tumor remains an area with many unanswered questions [15, 16].

Adipose tissue is increasingly recognized not merely as a fat storage site but as a significant regulatory role in tumor growth and metastasis [17, 18]. In colon cancer, abnormalities in the adipose tissue near the primary tumor may signify complex interactions between the tumor and its microenvironment [19, 20]. Previous studies have suggested that adipose tissue can influence cancer progression by secretion of adipokines and inflammatory mediators, impacting the tumor microenvironment [21]. This research aims to conduct an in-depth and structured examination of both imaging and genomic data from individuals with

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colon cancer to identify the correlation between unusual imaging characteristics of adipose tissue surrounding the primary tumor and the cancer's genomic profile.

Through this study, we aspire to discover novel biomarkers for early detection, deliver more customized data for deciding colon cancer treatments, and, ultimately, enhance patient survival rates. The clinical effectiveness of colon cancer treatments is frequently compromised by the recurrence of the tumor, making the ability to predict recurrence a critical measure for assessing patient outcomes [22]. Monitoring the clinical trajectory of patients will allow us to assess how anomalies in imaging when paired with genomic information, influence the likelihood of cancer recurrence. Such insights are anticipated to refine our predictive models for recurrence, offering healthcare professionals more precise prognostic information and, thus, laying a scientific foundation for creating tailored treatment regimens. Embarking on a multi-layered analysis that marries imaging with genomics represents a forward-thinking approach in colon cancer research [23]. By gaining a thorough understanding of the atypical imaging features of adipose tissue around the primary tumor, we aim to uncover the fundamental biological processes and molecular mechanisms, thereby seeding novel concepts and insights in the domain of oncology research.

By identifying the correlation between unusual imaging characteristics of adipose tissue surrounding the primary tumor and the genomic profile of the cancer, we aim to uncover new biomarkers for early detection and more personalized treatment strategies. This multi-layered analysis represents a forward-thinking approach in colon cancer research, offering potential for significant advancements in predicting and managing cancer recurrence, ultimately improving patient outcomes.

MATERIALS AND METHODS

Research design

This investigation is designed as a prospective cohort study, aiming to meticulously analyze the link between the unusual imaging features of adipose tissue near the colon cancer primary tumor, genomic data, and the likelihood of recurrence. Through an integration of imaging assessment, genomic sequencing, and ongoing patient follow-up, this study is poised to offer a detailed insight into the molecular attributes of colon cancer and evaluate recurrence risks over an extended period. Ethics approval was secured from the Ethics Committee of Tianjin Medical University (TY145726, 2022.1.21), safeguarding participant rights and welfare. Participants will be selected from the pool of colon cancer patients at our institution, with inclusion based on specified criteria. Participant profiles will be comprehensively developed, including data on age, sex, medical and family history.

Inclusion and discharge standards

Inclusion requirements consist of colon cancer patients aged 18 to 70 who have undergone surgical removal of the primary tumor and have had thorough systemic or localized imaging assessments (Using CT scanning and enhancement and MRI T1-weighted, T2 -weighted and enhancement sequences) to determine the tumor's origin, including the imaging of surrounding adipose tissue. Eligible patients must consent to participate, be able to comply with study procedures, sign an informed consent document, and provide surgical and blood samples for genomic study. An example diagram of the patient is shown in figure 1.

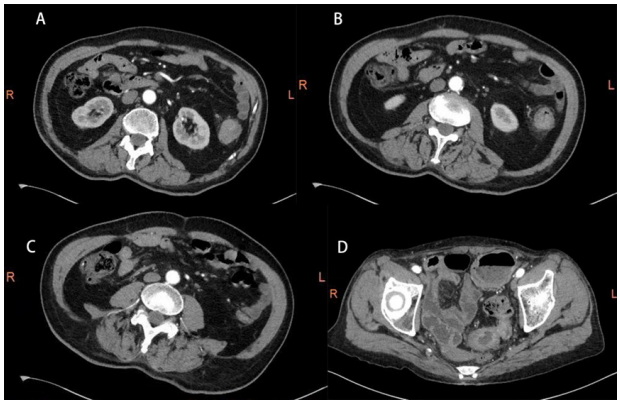

Figure 1. CT images of patients associated with **A, C** (imaging normal group), **B, D** (imaging abnormal group).

Exclusion criteria include individuals under 18 or over 70, those with other malignant tumors, significant organ dysfunction (heart, liver, kidney), severe infections or immune disorders, or any condition that might interfere with imaging assessments, such as significant post-surgical scarring impacting the evaluation of adjacent adipose tissue.

Grouping situation

Patients will be categorized based on the imaging characteristics of the adipose tissue around the primary tumor into groups with either normal or abnormal imaging findings. Abnormalities may encompass changes in fat density, irregular enhancement, among others. This classification facilitates the comparison of genomic and clinical outcomes across both patient sets.

Interventions

The study interventions involve both imaging evaluations and genomic analyses.

For imaging, all participants will undergo scans to assess the adipose tissue around the colon cancer

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primary tumor, with procedures like CT and MRI evaluated by seasoned radiologists for result accuracy and consistency. All CT images were acquired from a CT scanner (SOMATOM Definition Edge, Siemens, Germany). The contrast agent was Omnipaque (Norway). The scanning software was Syngo.via. All MRI images were obtained from a 3T MRI scanner (MAGNETOM Skyra, Siemens, Germany) and the contrast agent was Gadovist (Germany). Scanning software was AW Server.

The genomic analysis will entail extracting DNA from provided surgical and blood samples for comprehensive genomic sequencing, including whole -exome sequencing, mutation analysis, copy number variation assessments, etc., linking these genomic insights with imaging observations to uncover the molecular profile of the peritumoral adipose tissue.

Sample type and DNA extraction

For this genomic study, both blood and tissue samples were utilized. The surgical samples were collected from peritumoral adipose tissue during surgical procedures, while blood samples were collected through standard venipuncture techniques. The DNA extraction from these samples was performed using the QIAamp DNA Mini Kit (Qiagen, Germany) for tissue samples and the QIAamp DNA Blood Mini Kit (Qiagen, Germany) for blood samples. These kits are widely recognized for their efficiency and reliability in obtaining high-quality genomic DNA.

DNA extraction process

The extraction process began with the homogenization of tissue samples using a mechanical homogenizer. The homogenized tissue and blood samples were then subjected to lysis using proteinase K and lysis buffer provided in the respective kits. After the lysis step, the samples were incubated at 56°C to ensure complete cell lysis and protein digestion. The lysates were then applied to the QIAamp spin columns, where DNA was selectively bound to the silica membrane. Following several wash steps to remove contaminants, the DNA was eluted in a low-salt buffer and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

Whole-exome sequencing

The extracted DNA was then prepared for whole-exome sequencing (WES) using the Agilent SureSelect Human All Exon V7 kit (Agilent Technologies, USA). This kit captures the exonic regions of the genome, enabling the identification of coding variants. The DNA libraries were prepared according to the manufacturer's protocol, which included DNA fragmentation, end repair, adapter ligation, and PCR amplification. The libraries were then hybridized with biotinylated RNA baits specific to the exonic regions, and the captured DNA was purified using streptavidin-coated magnetic beads. The purified libraries were sequenced on the Illumina HiSeq 2500 platform (Illumina, USA), providing high-throughput, paired-end reads. This platform is known for its accuracy and depth of coverage, ensuring comprehensive detection of genomic variations.

Data analysis and filtering

The sequencing data were processed using a bioinformatics pipeline that included alignment to the human reference genome (hg19) using the Burrows-Wheeler Aligner (BWA) and variant calling using the Genome Analysis Toolkit (GATK). The initial variants underwent rigorous filtering to exclude common polymorphisms (using databases such as dbSNP, 1000 Genomes, and ExAC), low-quality variants, and artifacts.

To focus on the most relevant genomic alterations, the following filtering criteria were applied: 1. Functional Annotation: Variants were annotated using ANNOVAR, focusing on those predicted to affect protein function (e.g., nonsynonymous SNVs, frameshift indels, splice site variants). 2. Pathway Analysis: Identified variants were further analyzed using pathway enrichment tools (such as DAVID and Reactome) to prioritize genes in pathways relevant to tumor biology and adipose tissue function. 3. Frequency and Impact: Variants were filtered based on their frequency in the population (rare variants with a minor allele frequency <1%) and predicted impact on gene function (using tools such as SIFT, PolyPhen-2, and Mutation Taster).

Linking genomic data with imaging findings

Finally, to relate the genomic findings with imaging observations, two common genes were identified that showed a significant correlation with imaging biomarkers of peritumoral adipose tissue characteristics. This integrative analysis involved statistical methods such as correlation analysis and machine learning techniques to identify genes whose expression or mutation status was associated with specific imaging features, thus uncovering potential molecular drivers of the observed phenotypic changes.

Observation indicators

Evaluation of imaging will cover both quantitative and qualitative aspects such as tissue density, enhancement, and structure near the primary tumor.

Genomic findings will detail molecular features like mutations and copy number variations in genes related to colon cancer.

Recurrence likelihood will be monitored through patient follow-up, noting the timing and location of any tumor recurrence.

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Statistical analysis

Data analysis will employ the SPSS 25.0 software for descriptive statistics on patient demographics, imaging features, and genomic details. Continuous variable comparisons between groups will utilize t-tests or Mann-Whitney U tests, while chi-square tests or Fisher's exact tests will be used for categorical data. Patient survival and differences between groups will be assessed with Kaplan-Meier curves and Log-rank tests, respectively. The Cox proportional hazards model will analyze factors influencing recurrence risk, including imaging and genomic characteristics.

RESULTS

Over the course of this study, we enrolled 50 colon cancer patients who fulfilled the inclusion criteria. This cohort comprised with 35 males and 15 females, aged between 35 and 65 years. Each participant underwent imaging studies of the adipose tissue encircling the primary colon cancer lesion and completed the genomic analysis process. Based on the imaging findings, participants were categorized into two groups: those with abnormal imaging characteristics (30 patients) and those with normal imaging characteristics (20 patients) (table 1). The two groups did not show differences in all characteristics (P>0.05).

Table 1. Patient clinical characteristics.

Group	Abnormal imaging group	Normal imaging group	p-value
age	55.2±7.3	58.5±6.8	>0.05
Male (number of cases/ percentage)	20 (66.7%)	15 (75%)	
Body mass index (kg/m²)	25.1±2.5	24.8±2.3	
Smoking history (yes/no)	15 (50%)	10 (50%)	
Drinking history (yes/no)	18 (60%)	12 (60%)	
High blood pressure (yes/no)	12 (40%)	10 (50%)	
Diabetes (yes/no)	10 (33.3%)	8 (40%)	
Glycated hemoglobin (%)	6.8±1.2	6.5±1.0	
Total cholesterol (mmoL/L)	5.2±0.8	5.1±0.7	
Triglycerides (mmoL/L)	1.9±0.5	1.8±0.4	
Low-density lipoprotein (mmoL/L)	2.7±0.4	2.5±0.3	
Phospholipid protein A1 (g/L)	1.2±0.3	1.1±0.2	
Phospholipid protein B (g/L)	1.5±0.4	1.4±0.3	
Lipoprotein (mg/dL)	120±15	118±12	
Tumor stage (I/II/III/IV)	5 (16.7%) / 10 (33.3%) / 10 (33.3%) / 5 (16.7%)	3 (15%) / 7 (35%) / 7 (35%) / 3 (15%)	
CEA level (ng/mL)	5.3 ± 1.5	4.8 ± 1.3	
CA19-9 level (U/mL)	37 ± 10	35 ± 9	

Imaging manifestations

In the group with abnormal imaging characteristics, a substantial majority of 25 patients (83.3%) displayed atypical variations in fat density adjacent to the tumor. Localized increases characterized these variations or decreases in fat density, suggesting normal adipose tissue architecture disruptions. In contrast, such changes were observed in only two individuals (10%) within the group with typical imaging characteristics, indicating a statistically significant difference with a p-value of < 0.0001 (figure 2a). Moreover, heterogeneous enhancement, a key hallmark in the abnormal imaging group, was evident in 20 patients (66.7%). This enhancement indicated areas of local uneven enhancement, suggesting potential pathological changes such as fibrosis or increased vascularity. This feature was notably absent in the normal imaging group, further underscoring the distinct imaging patterns associated with the abnormal group. The difference in heterogeneous enhancement between the two groups was statistically significant, with a p-value of < 0.0001 (figure 2b). Significant morphological alterations in the peritumoral adipose tissue were observed in 15 patients (50%) within the abnormal imaging group. These alterations included indistinct margins and nodularity, indicating structural disruptions likely related to tumor interaction or inflammatory processes. In stark contrast, the normal imaging group maintained essentially unchanged morphology, with well-defined adipose tissue structures. This difference in morphological alterations was statistically significant, with a p-value of <0.0001 (figure 2c). Furthermore, enhanced adipose tissue, suggestive of localized inflammatory processes or angiogenesis, was detected in 10 patients (33.3%) in the abnormal imaging group. This enhancement highlights the active pathological processes occurring in the peritumoral environment, which were absent in the normal imaging group, indicating no signs of such inflammatory or angiogenic activities. The difference in the presence of enhanced adipose tissue between the two groups was statistically significant, with a p-value of <0.0001 (figure 2d).

Genomic signature

Genomic analyses identified distinct molecular patterns in the adipose tissue surrounding the primary tumors. Seventeen different mutations were identified in the abnormal group of imaging results. The *TP53* gene (60%) and *KRAS* gene (50%) were significantly more frequent in the abnormal imaging group than in the normal imaging group (*TP53*: 0, *KRAS*: 0) (P<0.001). Meanwhile, gene mutation frequencies were notably lower in the normal imaging group, with the APC gene mutations present in 25% of cases (figure 3).

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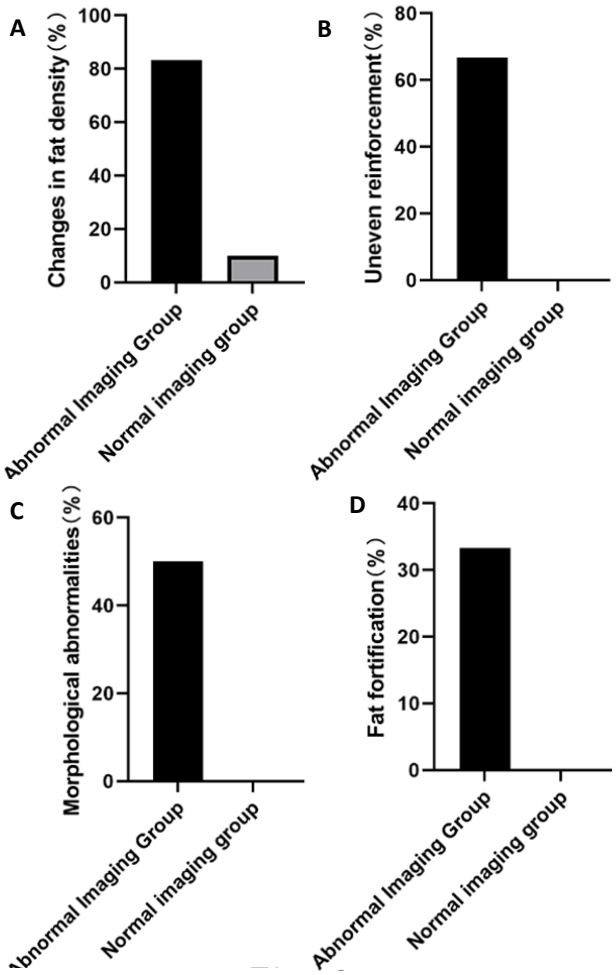

Figure 2. Changes of surrounding tumor fat density in groups with abnormal imaging characteristics and normal group.

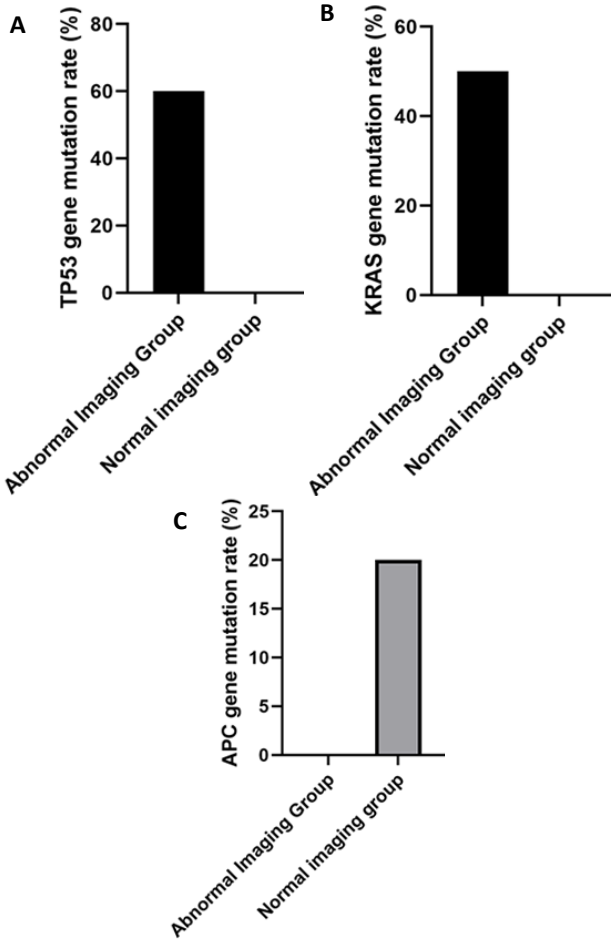

Figure 3. Gene mutation characteristics of abnormal imaging characteristics group and normal group.

Relapse probability

Ongoing patient follow-up indicated that 12 individuals in the abnormal imaging group experienced disease recurrence, compared to only 5 in the normal imaging group. Survival analysis revealed a significant difference in median recurrence-free survival times between the groups: 18 months in the abnormal imaging group versus 28 months in the normal imaging group (Log-rank test, P<0.05) (figure 4).

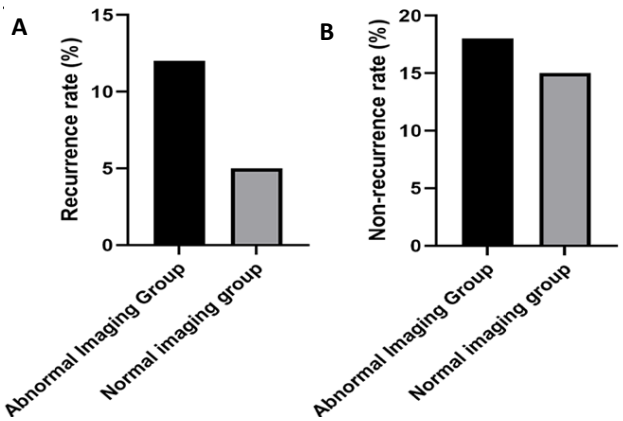

Figure 4. Statistics of the difference in survival time between the abnormal imaging characteristics and the normal groups.

Multi-factor analysis

In the Cox proportional hazards model analysis, abnormal imaging findings (HR=2.35, 95%CI: 1.12-4.94, P=0.024), certain gene mutations (such as *TP53* gene, HR=3.12, 95%CI: 1.42-6.85, P=0.004) was closely associated with colon cancer recurrence (table 2).

Table 2. Cox proportional hazards model analysis and association with clinical characteristics

variable	HR (hazard ratio)	95% CI	p
Abnormal imaging findings	2.35	1.12-4.94	0.024
TP53 gene mutation	3.12	1.42-6.85	0.004
Age (for each additional year)	1.08	1.01-1.15	0.032
Smoking history	1.78	0.89-3.56	0.105
Hypertension	1.95	1.08-3.51	0.027
Diabetes	2.1	1.15-3.84	0.015

DISCUSSION

The presence of altered fat density and heterogeneous enhancement observed in the group with abnormal imaging could indicate modifications within the adipose tissue's microenvironment near the primary tumor. Such changes are associated with prevalent mutations in genes like *TP53* and *KRAS*, as

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identified through our genomic analyses [24, 25]. It is speculated that these genetic alterations may drive changes in the tumor microenvironment, thereby influencing the appearance of surrounding adipose tissue. Furthermore, the interaction between these genetic mutations and the microenvironment could foster an environment conducive to tumor progression and metastasis [26, 27].

Our analysis revealed a notably higher recurrence rate among patients exhibiting abnormal imaging findings compared to those with normal imaging [28]. According to the Cox proportional hazards model, abnormalities in imaging and specific genetic alterations, such as mutations in the *TP53* gene, stand as independent predictors for the recurrence of colon cancer [29, 30]. This highlights the potential impact of certain genetic changes on the tumor's behavior, predisposing patients to a higher likelihood of recurrence. These findings suggest that the interplay between imaging characteristics and genetic profiles could serve as a more reliable indicator of prognosis than either factor alone.

By integrating imaging data with genomic insights, we can achieve a fuller understanding of colon cancer patient conditions. Those presenting both abnormal imaging characteristics and particular genetic mutations might necessitate more intensive monitoring and tailored therapeutic approaches [31]. For instance, patients harboring *TP53* gene mutations might benefit from more robust surveillance and treatments that target this specific mutation [32]. This approach could involve the use of personalized therapies designed to mitigate the effects of specific genetic alterations, potentially improving patient outcomes [33].

Additionally, the unusual imaging features of adipose tissue might serve as a non-invasive biomarker for identifying high-risk patients. This could facilitate earlier interventions and more aggressive treatment plans tailored to the individual's genetic makeup and tumor microenvironment. Overall, the integration of imaging and genomic data provides a promising avenue for enhancing the precision of colon cancer treatment strategies, ultimately aiming to reduce recurrence rates and improve patient survival.

Nevertheless, this study is not without its limitations, including a small sample size and a focus limited to patients from a single hospital. Future studies, especially those with larger samples and multi-center collaboration, are needed to corroborate our findings and extend their applicability to a wider patient demographic. In conclusion, our study provides new insights into the biological traits of adipose tissue surrounding the primary tumor in colon cancer patients by merging imaging and genomic data, thereby offering fresh perspectives for personalized treatment. This holistic approach to research paves the way for more accurate and individualized future treatment modalities for colon cancer.

In conclusion, our study highlights the significant interplay between imaging characteristics and genetic profiles in understanding and managing colon cancer. The presence of altered fat density and heterogeneous enhancement around the primary tumor, linked to mutations in genes like *TP53* and *KRAS*, suggests modifications within the adipose tissue's microenvironment that may contribute to tumor progression and recurrence. This integrated approach not only provides a comprehensive understanding of the tumor microenvironment but also identifies high-risk patients who may benefit from more intensive monitoring and tailored therapeutic strategies. Personalized treatments targeting specific genetic mutations, such as those in the *TP53* gene, hold promise for improving patient outcomes. Additionally, the unusual imaging features of adipose tissue could serve as non-invasive biomarkers for earlier interventions.

Ethical consideration: This study was approved by the Ethics Committee of Tianjin Medical University (TY145726, 2022.1.21).

Conflict of interest: The authors have no potential conflicts of interest to report relevant to this article.

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Author contributions: Q.W. and H.W. designed the study and performed the experiments, Z.W. collected the data, L.L. analyzed the data, Q.W. and H.W. prepared the manuscript. All authors read and approved the final manuscript.

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