

Establishment of a Standardized Rat Model of Chronic Radiation Proctitis and Preliminary Evaluation of Its Association with Tubulin

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**Establishment of a Standardized Rat Model of Chronic Radiation
Proctitis and Preliminary Evaluation of Its Association with Tubulin**

Title Page

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Abstract

Background: Chronic radiation proctitis, a major complication of pelvic radiotherapy, lacks effective treatments and a standardized animal model that recapitulates its late fibrotic and angiogenic pathologies.

Aim: This study aimed to establish a chronic radiation injury model resembling the progression of human radiation proctitis. Serial endoscopic observations were conducted to examine long-term rectal mucosal changes in rats post-irradiation, and the relationship with cytoskeletal proteins was preliminarily assessed.

Methods: The rats were randomly divided into 12 groups, including radiation dose groups of 12.5–37.5 Gy and a control group. A single-batch irradiation method was used to establish the model. The degree of rectal injury and progression over six months were assessed using endoscopic observations, the modified Vienna rectal endoscopic scoring system, and histological methods.

Results: Below 20 Gy, mucosal injury was negligible following resolution of acute inflammation. 20–35 Gy, marked capillary dilation appeared around the eighth week post-irradiation, ultimately resulting in persistent radiation-induced proctitis. At 20 Gy, a sharp decrease in cytoskeletal proteins, particularly tubulin, was observed. This reduction correlated with the eventual development of chronic radiation injury in the 20–35 Gy groups. Moreover, VEGF was upregulated in the chronic phase.

Conclusions: A single rectal irradiation of 25 Gy is recommended to simplify the

model establishment process and achieve successful induction of chronic radiation proctitis in rats while ensuring survival. When assessing pharmacological interventions for radiation proctitis, the observation period should be extended to at least eight weeks.

Keywords

Chronic radiation proctitis; Endoscopic views; Endoscopic scoring; Tubulin; Rat model

Introduction

Radiotherapy is frequently used to treat pelvic malignancies, with approximately 300,000 patients worldwide undergoing pelvic irradiation annually^[1]. Among radiation-induced complications, rectal injury is the most common. Radiation proctitis (RP) can be classified as acute or chronic according to symptom onset and the severity of tissue damage^[2]. Acute radiation proctitis(ARP) typically manifests as tenesmus and diarrhea, occasionally accompanied by mild bleeding^[3], and generally resolves spontaneously. In contrast, chronic radiation proctitis (CRP) has a later onset and tends to persist^[4]. The most frequent symptom of CRP is rectal bleeding, followed by fibrotic changes leading to strictures^[5]. These symptoms significantly reduce patient quality of life, and currently, there is no effective treatment. Thus, CRP remains an important focus of both clinical and basic research.

Previous studies on radiation proctitis have predominantly utilized acute radiation injury models^[6, 7], which do not adequately replicate the pathophysiology of chronic injuries resulting from radiotherapy. Therefore, establishing an animal model

that closely mimics human CRP progression is essential. Several modeling approaches have been reported, ranging from high-dose^[8] to low-dose^[9] irradiation, and from single-dose exposure to multiple fractionated low-dose regimens^[10, 11]. Although these methods induce varying degrees of rectal injury, a standardized animal model and evaluation system for chronic radiation injury remains lacking. In this study, long-term mucosal changes in rat rectum were monitored by in vivo endoscopy, combined with acute and chronic symptom scoring, histopathological assessments, and molecular biological analyses for comprehensive evaluation.

Methods and Materials

Animals and Irradiation Procedure

All animal experiments were reviewed and approved by XXXX. Male Sprague-Dawley rats (8 weeks old, specific pathogen-free) weighing 250 ± 20 g were used. The rats were acclimated under standard laboratory conditions with free access to food and water for one week prior to irradiation.

The rats were randomly divided into 12 groups (eight rats per group), including radiation dose groups of 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35, and 37.5 Gy, as well as a control group. Rats were immobilized in transparent plastic holders, and irradiation was simultaneously delivered to five rats placed on a wooden board to enhance efficiency (Fig. 1). X-ray irradiation was administered using a linear accelerator with a rectangular collimated field. Except for the rectal region, the pelvic area was shielded using a 3 cm thick lead block to minimize exposure of surrounding tissues.

Endoscopic Observation and Scoring

The rats were fasted for 12 hours before colonoscopy but had free access to water to facilitate clear visualization. Anesthesia was induced by inhalation of isoflurane, and the rats were placed prone on a thermostatic surgical platform (37 °C). The tail was gently lifted to straighten the rectum. The anus and endoscope were lubricated with paraffin oil, and a cholangioscope (xxxx,diameter 2.9 mm) was carefully inserted along the anterior rectal wall. During insertion, sterile saline was infused through the instrument channel to improve visibility and prevent intestinal spasms. If resistance occurred, the scope was withdrawn and repositioned at a different angle to avoid mucosal damage or perforation. During withdrawal, the distal colon and entire rectum were thoroughly examined, and both images and videos were recorded.

Sequential endoscopic examinations were conducted at 2, 4, 6, and 8 weeks and at 3, 4, 5, and 6 months after irradiation. These examinations allowed continuous monitoring of mucosal progression from the acute to chronic phases. Findings were evaluated using an endoscopic scoring system developed by modifying the Vienna Rectoscopy Score (VRS)^[12] specifically for rats (Table 1).

Euthanasia

At three months post-irradiation, four rats from each group were randomly euthanized, and the remaining rats were euthanized at six months. Blood samples were collected, allowed to stand at room temperature for 30 minutes, and centrifuged at 3,000 rpm for 10 minutes. Serum supernatant was stored at –80 °C. Rectal tissue located 3 cm above the anus was excised, rinsed, and rapidly frozen in liquid nitrogen for storage at –

80 °C. Samples intended for Western blot analysis were preserved in liquid nitrogen, whereas tissues for histopathological analysis were fixed in 4% paraformaldehyde.

Histopathological Analysis

Rectal specimens were fixed in 4% paraformaldehyde and stained with hematoxylin-eosin (HE) and Masson's trichrome. Tissue sections (4 µm thick) were prepared using a rotary microtome (Leica, Germany), placed onto glass slides, and stained according to standard protocols. Sections were observed under a light microscope (DM2500, Leica) following established methods. Collagen fibers, nuclei, and cytoplasm were stained blue, dark purple, and red/pink, respectively, by Masson's trichrome staining. Histological changes were quantified using a Radiation Injury Score (RIS) adapted from Langberg et al.^[13], which assesses mucosal ulceration, inflammatory cell infiltration, edema, vascular narrowing, and submucosal fibrosis.

Immunofluorescence Analysis

Rectal tissue sections were fixed in 4% paraformaldehyde at room temperature for 30 minutes, permeabilized with 0.1% Triton X-100 for 10 minutes, and blocked with 5% BSA for 30 minutes to prevent nonspecific binding. Sections were incubated overnight at 4 °C in a humidified chamber with anti-VEGF primary antibody (1:100 dilution). After washing, a Cy3-conjugated anti-rabbit IgG (1:200 dilution) was applied for 1 hour at room temperature in darkness. Finally, nuclei were counterstained with DAPI (1:1000 dilution) for 5 minutes at room temperature, protected from light.

Western Blot Analysis

Rectal tissues were lysed, and total protein was extracted and quantified. Equal protein amounts were mixed with loading buffer, separated by 10% SDS-PAGE, and transferred onto PVDF membranes. Membranes were blocked in 5% skim milk for 1 hour, incubated overnight at 4 °C with primary antibodies, followed by incubation with HRP-conjugated secondary antibodies at room temperature for 1 hour. Protein bands were visualized using an ECL detection kit, and images were captured with a Tanon 5200 imaging system.

Results

Effects of Different Radiation Doses on Rats

In this study, mortality was minimal in the 12.5–20 Gy groups. In contrast, mortality increased progressively with higher radiation doses (20–35 Gy), and extensive deaths occurred in the 37.5 Gy group during the acute inflammatory phase. Six-month survival analysis revealed that higher radiation doses resulted in earlier mortality, peaking between 120–160 days post-irradiation(Fig. 2E). Necropsy demonstrated varying degrees of rectal obstruction extending into the colon, causing difficulties in defecation. Concurrent complications included urinary retention, bladder distension, and highly concentrated urine.

Endoscopic findings were classified into three severity levels:

Mild (12.5–20 Gy): Mild mucosal hyperemia, edema, and occasional pinpoint bleeding.

Moderate (22.5–27.5 Gy): Diffuse mucosal hyperemia and edema, thickened mucosa, swollen folds covered with fibrinous white exudate, and increased mucosal fragility

with easy bleeding upon contact.

Severe (30–37.5 Gy): Extensive mucosal hyperemia and edema causing luminal narrowing, widespread active bleeding with minimal contact, and visible ulceration under endoscopy(Fig. 2A).

Lesions were graded based on capillary dilation, mucosal hyperemia, ulceration, stenosis, and necrosis, and scores were summed. Previous studies by Northway et al. indicated pathological injury scores peak between 7–15 days post-irradiation^[14, 15]. Accordingly, a dose versus 2-week endoscopic score curve was generated (Fig. 2C). Mucosal injury was mild at doses below 20 Gy and progressively worsened with increasing radiation dose. At three months, the 15 Gy group displayed mild injury, showing atrophic mucosal epithelium and slight submucosal fibrosis thickening by HE staining, allowing mucosal self-repair. Higher doses induced significant submucosal fibrosis, arterial wall fibrosis, glandular disorganization, severe rectal wall rigidity, widespread mucosal ulceration, and complete glandular destruction. Full-thickness necrosis occurred at 35 Gy(Fig. 2B). RIS trends were consistent with endoscopic scores. Because groups differing by 2.5 Gy showed similar histopathological changes, representative results are presented at 5 Gy intervals(Fig. 2D).

VEGF Expression Increases During the Chronic Phase of Radiation Proctitis

To evaluate the localization and temporal changes of VEGF expression during radiation proctitis progression, immunofluorescence staining was performed on rat rectal tissues at 2 weeks and 3 months post-irradiation. At 2 weeks, VEGF expression

increased moderately in irradiated groups compared to controls, but no significant expression appeared within the mucosal layer. At 3 months, VEGF expression was almost undetectable in the 15 Gy group. In contrast, prominent VEGF expression occurred within fibrotic mucosal tissue and the lumen in the 20–35 Gy groups(Fig. 3). In these groups, VEGF levels increased not only during the acute phase but also markedly during the chronic phase, particularly in the mucosal layer. These findings suggest that sustained elevation of VEGF in rectal tissues might serve as a characteristic feature of CRP.

Endoscopic Time-Course Changes in Rats Exposed to Different Radiation Doses

Based on sequential endoscopic observations, responses could be divided into three categories:Mild (12.5–17.5 Gy, e.g., 15 Gy): Mild mucosal edema and pinpoint bleeding were noted during the acute phase. Later, inflammation resolved and mucosal healing persisted up to 6 months(Fig. 4A). Moderate (20–25 Gy, e.g., 25 Gy): After partial resolution of acute inflammation, symptoms slightly improved. However, at approximately 8 weeks, various degrees of capillary dilation emerged due to abnormal tissue remodeling. Endoscopy performed between 3–6 months showed luminal narrowing, pale mucosa, and rigid rectal walls(Fig. 4B).Severe (27.5–37.5 Gy, e.g., 35 Gy): Severe mucosal epithelial shedding caused pronounced edema, inflammatory stenosis, and active mucosal bleeding. At 8 weeks, endoscopy revealed red, spiderweb-like vascular patterns. Progressive fibrosis resulted in severe luminal narrowing(Fig. 4C). Unlike the 25 Gy group, rats irradiated with 35 Gy maintained persistently high endoscopic scores(Fig. 4D). Continuous endoscopic monitoring at

doses above 35 Gy was impractical due to high mortality.

Endoscopic observations revealed extensive capillary dilation around the eighth week post-irradiation in groups exposed to doses exceeding 20 Gy. At 6 months, these groups exhibited persistent mucosal injury, failing to heal, and eventually developed CRP. Conversely, rats irradiated below 20 Gy exhibited resolution of inflammation and mucosal recovery.

Radiation-Induced Disruption of α -Tubulin Impairs Self-Repair in Rats

To investigate potential factors underlying these differences, rectal tissues collected at 2 weeks post-irradiation were analyzed. Western blot analysis showed a sharp decline in α -tubulin expression at 20 Gy, whereas expression remained high in the 15 Gy group (Fig. 4E). This result aligned with endoscopic findings indicating chronic injury occurred at doses exceeding 20 Gy. Radiation above 20 Gy severely disrupted the microtubule cytoskeleton, impairing mitosis and intracellular transport. These effects may explain why rats exposed to high-dose irradiation failed to achieve self-repair, leading to CRP.

Discussion

Fractionated irradiation was not included as a separate group in this study. Previous reports indicate fractionated radiotherapy aims primarily to reduce radiation damage [16, 17]. For modeling chronic proctitis, single high-dose irradiation produces more severe injury than cumulative fractionated low-dose radiation. ARP is mainly characterized by mucosal inflammatory infiltration, while the chronic phase predominantly involves regenerative and fibrotic changes^[5]. In this study, a single

high-dose irradiation effectively established a CRP rat model, closely resembling clinical manifestations observed in patients post-radiotherapy. Therefore, a single high-dose irradiation protocol is recommended to simplify modeling, ensure consistent radiation exposure, and minimize variability.

Based on sequential endoscopic and histopathological findings, radiation doses of 20–35 Gy were sufficient to induce chronic rectal injury. A dose of 25 Gy is recommended for pharmacological intervention studies. This dose achieves significant disruption of microtubule structures, allowing clear distinction between therapeutic effects and the rats' inherent repair capacity while maintaining adequate survival rates. Extensive capillary dilation observed at 8 weeks resulted from abnormal tissue remodeling, failing to restore normal structure and function, thus causing persistent CRP. For evaluating therapeutic interventions, observation periods should extend to at least 8 weeks to accurately assess improvements in chronic symptoms. We propose that preclinical studies evaluating therapeutic interventions for radiation proctitis should extend the experimental endpoint to at least 8 weeks. This is critical for assessing efficacy against chronic symptoms, as acute endoscopic findings such as edema and inflammatory cell infiltration typically resolve spontaneously. Consequently, analyzing inflammatory markers during the resolution phase of acute edema may lead to inaccurate conclusions regarding a drug's long-term effects.

Incorporating endoscopic observation of rectal mucosa as an efficacy indicator provides a more intuitive evaluation than relying solely on histopathological findings^[14, 18]. Endoscopy enables visualization of both the rectal and distal colonic

segments, facilitating continuous monitoring of injury progression and repair without animal euthanasia. Compared with histological evaluation using post-mortem tissue sections, endoscopic assessment offers more comprehensive insights into mucosal changes. During preliminary experiments, ultra-thin gastroscopes with gas insufflation were tested. However, many animals died due to pre-existing rectal obstruction combined with excessive colonic distension. In this study, a cholangioscope with a small-diameter shaft was employed, allowing safe passage through obstructed rectal segments. Saline infusion replaced gas insufflation, and no animal deaths occurred during endoscopy. Thus, we recommend using a cholangioscope for in vivo longitudinal monitoring due to its safety, effectiveness, and operational simplicity.

Zeng^[19] previously reported enrichment of angiogenesis-related genes at 8 weeks post-irradiation, while acute-phase genes primarily involved DNA damage responses. In this study, transient elevation of VEGF occurred during the acute phase, whereas chronically irradiated rat rectal mucosa displayed markedly increased VEGF expression, differing significantly from the acute phase. Hypoxia-induced VEGF overexpression promotes pathological angiogenesis and fibrosis, creating a vicious cycle that sustains chronic pathological progression. These findings suggest anti-VEGF therapies might alleviate symptoms by inhibiting pathological angiogenesis.

Previous studies indicate radiation can disrupt the cytoskeletal structure^[20, 21]. To explore the relationship between chronic injury from doses exceeding 20 Gy and

cytoskeletal proteins, Western blot analysis was performed. Rats irradiated with 20–35 Gy exhibited significantly reduced cytoskeletal proteins compared to the 15 Gy group, especially tubulin. Our ongoing research demonstrates that early intervention post-irradiation can preserve cytoskeletal integrity, promoting recovery from radiation proctitis. Restoration of cytoskeletal structure could thus become an evaluation parameter in therapeutic studies of RP. For studies investigating chronic radiation proctitis, especially in settings where endoscopic expertise is limited, we demonstrate that Western blot (WB) analysis of α -tubulin expression in rectal tissue serves as a reliable surrogate marker. This allows for the effective exclusion of animals that did not develop chronic injury, thereby ensuring the accuracy and rigor of subsequent drug efficacy experiments.

Conclusions

We recommend a single 25 Gy rectal irradiation dose to create persistent, non-healing injury while ensuring adequate survival, thus successfully establishing a CRP model in rats. For pharmacological intervention studies, the observation period should extend to at least eight weeks to accurately assess therapeutic efficacy.

References

- [1] Andreyev H J. Gastrointestinal problems after pelvic radiotherapy: the past, the present and the future[J]. Clin Oncol (R Coll Radiol), 2007, 19(10): 790-799.
- [2] Dahiya D S, Kichloo A, Tuma F, et al. Radiation Proctitis and Management Strategies[J]. Clin Endosc, 2022, 55(1): 22-32.

- [3] Esswein K, Ninkovic M, Kröpfl V, et al. Radiofrequency ablation: Solution for a long-time therapeutic dilemma of chronic radiation proctitis?[J]. *Endosc Int Open*, 2025, 13: a26051079.
- [4] Smirnov A V, Sychev V I, Kuznetsova S M, et al. Management of radiation-induced proctitis using submucosal endoscopic injections of autologous adipose-derived stromal vascular fraction: a case report[J]. *Stem Cell Res Ther*, 2024, 15(1): 410.
- [5] Mahmood S, Bollipo S, Steele S, et al. It's All the RAVE: Time to Give up on the "Chronic Radiation Proctitis" Misnomer[J]. *Gastroenterology*, 2021, 160(3): 635-638.
- [6] Gao J, Li Y, Chen J, et al. Emodin ameliorates acute radiation proctitis in mice by regulating AKT/MAPK/NF- κ B/VEGF pathways[J]. *Int Immunopharmacol*, 2024, 132: 111945.
- [7] Duan X, Cai H, Hu T, et al. Ginsenoside Rg3 treats acute radiation proctitis through the TLR4/MyD88/NF- κ B pathway and regulation of intestinal flora[J]. *Front Cell Infect Microbiol*, 2022, 12: 1028576.
- [8] Jee J, Park J H, Im J H, et al. Functional recovery by colon organoid transplantation in a mouse model of radiation proctitis[J]. *Biomaterials*, 2021, 275: 120925.
- [9] Ashcraft K A, Miles D, Sunday M E, et al. Development and Preliminary Evaluation of a Murine Model of Chronic Radiation-Induced Proctitis[J]. *Int J Radiat Oncol Biol Phys*, 2018, 101(5): 1194-1201.
- [10] Wang J, Kulkarni A, Chintala M, et al. Inhibition of protease-activated receptor 1 ameliorates intestinal radiation mucositis in a preclinical rat model[J]. *Int J Radiat Oncol Biol Phys*, 2013, 85(1): 208-214.
- [11] Leite C H B, Lopes C D H, Leite C, et al. A Novel Murine Model of a High Dose Brachytherapy-Induced Actinic Proctitis[J]. *Front Oncol*, 2022, 12: 802621.

- [12] Goldner G, Tomicek B, Becker G, et al. Proctitis after external-beam radiotherapy for prostate cancer classified by Vienna Rectoscopy Score and correlated with EORTC/RTOG score for late rectal toxicity: results of a prospective multicenter study of 166 patients[J]. *Int J Radiat Oncol Biol Phys*, 2007, 67(1): 78-83.
- [13] Langberg C W, Waldron J A, Baker M L, et al. Significance of overall treatment time for the development of radiation-induced intestinal complications. An experimental study in the rat[J]. *Cancer*, 1994, 73(10): 2663-2668.
- [14] Northway M G, Scobey M W, Geisinger K R. Radiation proctitis in the rat. Sequential changes and effects of anti-inflammatory agents[J]. *Cancer*, 1988, 62(9): 1962-1969.
- [15] Korkut C, Asoglu O, Aksoy M, et al. Histopathological comparison of topical therapy modalities for acute radiation proctitis in an experimental rat model[J]. *World J Gastroenterol*, 2006, 12(30): 4879-4883.
- [16] Lo Y, Taylor J M, McBride W H, et al. The effect of fractionated doses of radiation on mouse spinal cord[J]. *Int J Radiat Oncol Biol Phys*, 1993, 27(2): 309-317.
- [17] Griffin R J, Ahmed M M, Amendola B, et al. Understanding High-Dose, Ultra-High Dose Rate, and Spatially Fractionated Radiation Therapy[J]. *Int J Radiat Oncol Biol Phys*, 2020, 107(4): 766-778.
- [18] Doi H, Kamikonya N, Takada Y, et al. Efficacy of polaprezinc for acute radiation proctitis in a rat model[J]. *Int J Radiat Oncol Biol Phys*, 2011, 80(3): 877-884.
- [19] Zeng Q, Cheng J, Wu H, et al. The dynamic cellular and molecular features during the development of radiation proctitis revealed by transcriptomic profiling in mice[J]. *BMC Genomics*, 2022, 23(1): 431.

- [20] Barjaktarovic Z, Schmaltz D, Shyla A, et al. Radiation-induced signaling results in mitochondrial impairment in mouse heart at 4 weeks after exposure to X-rays[J]. PLoS One, 2011, 6(12): e27811.
- [21] Zheng Q, Liu Y, Zhou H J, et al. X-ray radiation promotes the metastatic potential of tongue squamous cell carcinoma cells via modulation of biomechanical and cytoskeletal properties[J]. Hum Exp Toxicol, 2015, 34(9): 894-903.

Table 1. Modified rectoscopic scoring system for rats

Score	Endoscopic Feature
Telangiectasia	
0 points	None
1 point	Scattered isolated vessels
2 points	Multiple telangiectasias
3 points	Diffuse telangiectatic networks
Congested mucosa	
0 points	None
1 point	Mild hyperemia or edema
2 points	Diffuse hyperemia
3 points	contact bleeding
4 points	Spontaneous bleeding
Ulceration	
0 points	None
1 point	Single small ulcer or Superficial ulcer
2 points	Multiple small ulcers or confluent patchy ulcers
3 points	Deep ulcer
4 points	Deep ulceration leads to fistula or perforation
Stricture	
0 points	None
1 point	> 2/3 of the diameter of the rectum
2 points	1/3-2/3 of the diameter of the rectum
3 points	<1/3 of the diameter of the rectum
4 points	entirely shut
necrosis	
0 points	None
1 point	Extensive necrosis

Figure Captions

Fig. 1 Irradiation setup showing rat fixation and shielding

The yellow rectangle indicates the irradiation area, while the red area represents the rectal region after tissue shielding.

Fig. 2 Effects of different radiation doses on rats

(A) Endoscopic appearance of rat rectum at 2 weeks post-irradiation across different dose groups;

(B) HE and Masson staining of rectal tissues at 3 months;

(C) Dose–endoscopic score curve;

(D) Dose–RIS curve;

(E) Survival curve.

Fig. 3 Immunofluorescence staining for VEGF in rat rectum at 2 weeks and 3 months post-irradiation

Fig. 4 Endoscopic time-course changes in rats exposed to different radiation doses

(A) Sequential endoscopic findings in the 15 Gy group;

(B) Sequential endoscopic findings in the 25 Gy group;

(C) Sequential endoscopic findings in the 35 Gy group;

(D) Time–endoscopic score curves for 15, 25, and 35 Gy groups;

(E) Western blot analysis of rectal cytoskeletal proteins at 2 weeks;

(F) β -actin expression across dose groups;

(G) α -tubulin expression across dose groups.

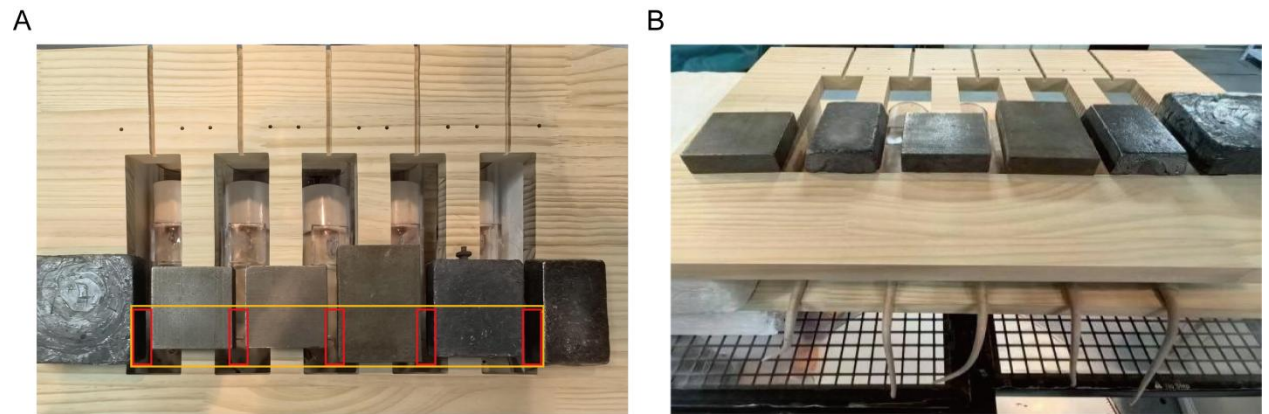


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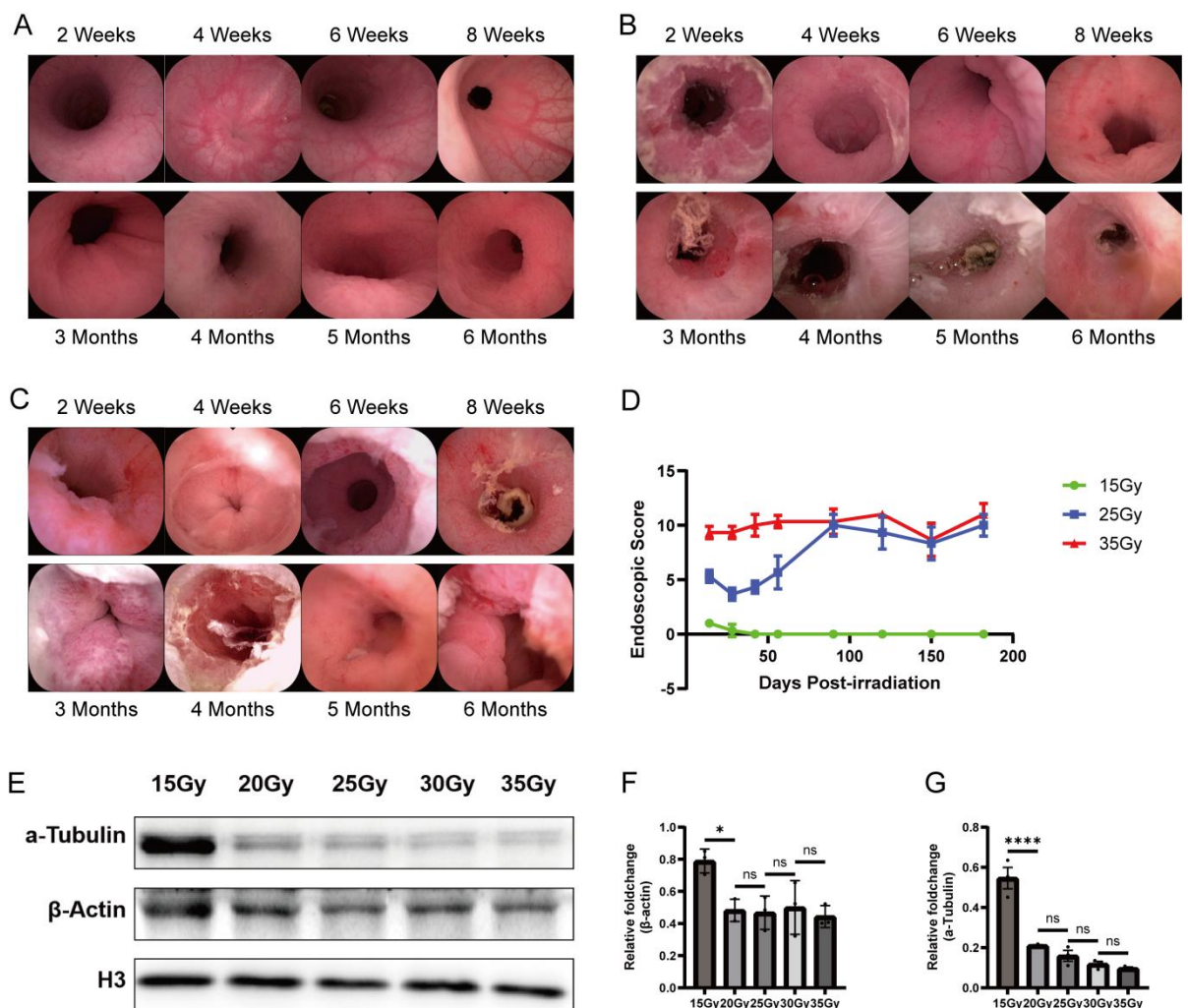


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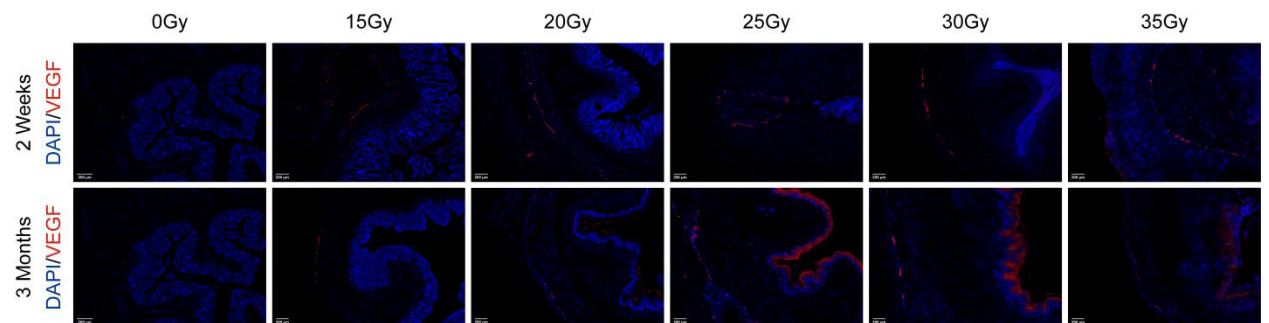


Fig. 3 Immunofluorescence staining for VEGF in rat rectum at 2 weeks and 3 months post-irradiation

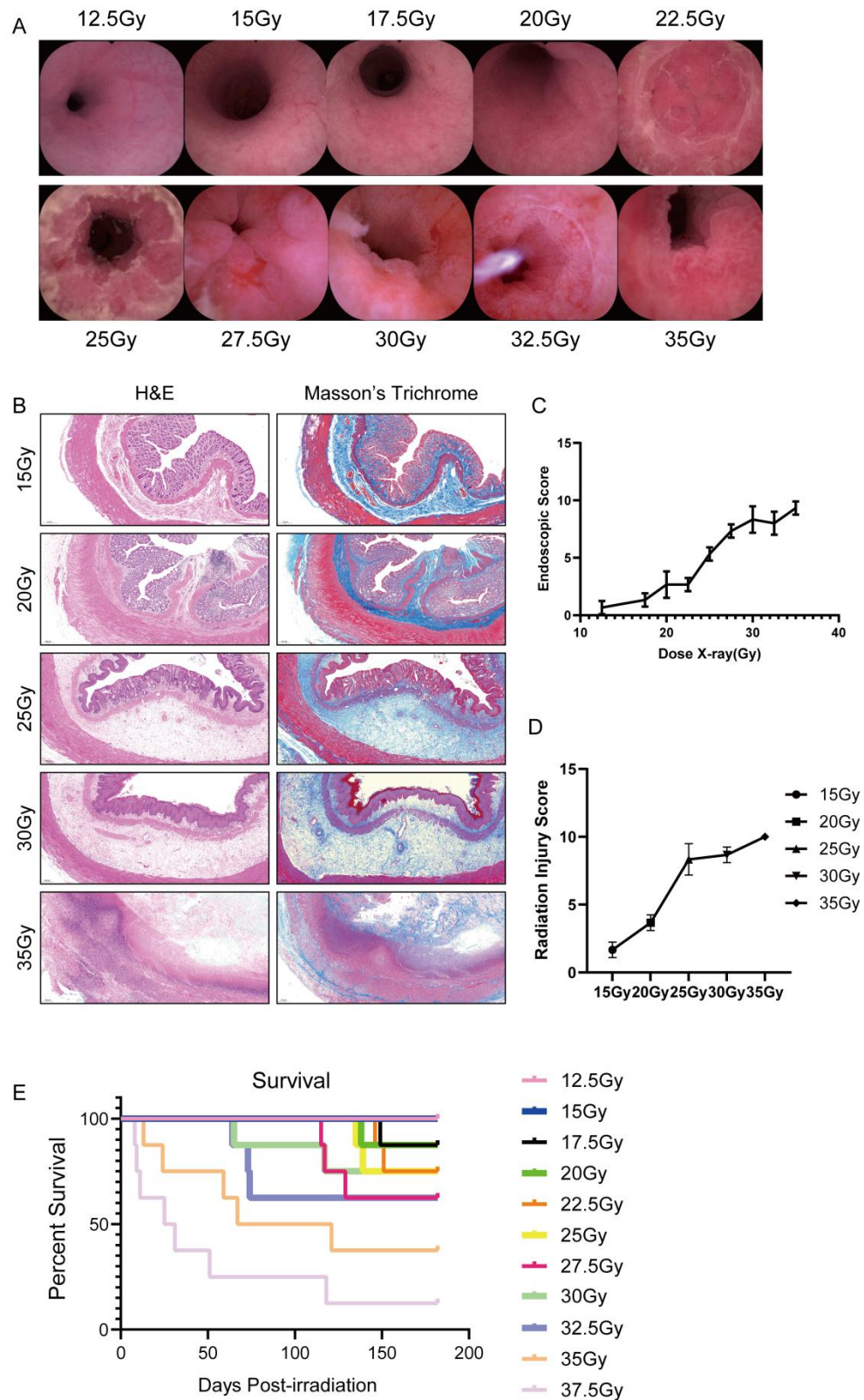


Fig. 4 Endoscopic time-course changes in rats exposed to different radiation doses