Experimental Research

ANNALS OF MEDICINE & SURGERY

OPEN

# KRAS-mutant colorectal cancer cell lines cause a prothrombotic state through the upregulation of thrombin: experimental study

Duogang Xu, MD[a,b], Changkang Liao, MD[a,b], Jing Tan, MD[a,b,*]

**Background:** The KRAS genotype status is strongly associated with a prothrombotic state in colorectal cancer, and hypercoagulability and cancer-related thrombosis are among the significant events leading to poor prognosis. However, this correlation has not been confirmed at the cellular level. This study aimed to assess the maximum platelet aggregation rate and thrombin expression induced by colorectal cancer cells under different KRAS genotypes.

**Materials and methods:** Platelet aggregation rate assay and western blotting analysis were used to detect platelet aggregation and thrombin expression induced by four colorectal cancer cells with different KRAS genotypes, including RKO, HCT116, SW480, and SW620. FVIIa/tissue factor and thrombin inhibitors were added to explore changes in platelet aggregation rates induced by colorectal cancer cells and the association between KRAS genotype status and hypercoagulable state.

**Results:** KRAS-mutant cells were more likely to increase maximal platelet aggregation, with RKO, HCT116, SW480, and SW620 inducing 34.7%, 55.4%, 44.4%, and 63.8% of platelet aggregation, respectively. The maximum platelet aggregation rate was higher in the metastatic rectal cancer tumour strain SW620 than in the primary rectal cancer strain SW480. RKO cells had lower thrombin expression than the other three cells. Furthermore, the addition of thrombin inhibitors caused a more significant decrease in the platelet aggregation rate in KRAS-mutant cell lines compared to KRAS wild-type cell lines.

**Conclusion:** Compared to KRAS wild-type colorectal cancer cells, KRAS-mutant colorectal cancer cell lines were more likely to be hypercoagulable through the upregulation of thrombin expression, which was mainly achieved through the TF-thrombin pathway.

**Keywords;** colorectal cancer cell, KRAS, platelet aggregation rate, tissue factor

## Introduction

The second leading cause of cancer-related mortality globally is colorectal cancer, which is also the third most prevalent cancer overall[1]. In 1865, the famous French doctor Armand Trousseau discovered the link between cancer and thrombosis; he defined superficial thrombophlebitis as an early indicator of concealed visceral malignancy[2]. Colorectal cancer is no exception and is reported to have the second-highest incidence of thrombotic lesions among several common cancers[3]. Hypercoagulability is associated with clinical progression and prognosis in patients with colorectal cancer. Many coagulation abnormalities, such as thrombocytosis, hyperfibrinogen, and elevated D-dimer levels, are associated with the progression or poor prognosis of colorectal cancer[4].

### HIGHLIGHTS

- Assessed platelet aggregation and thrombin expression with different KRAS genotypes.
- KRAS-mutant cells were more likely to increase maximal platelet aggregation.
- Thrombin inhibitors significantly decreased the platelet aggregation rate.
- KRAS mutants were hypercoagulable through upregulated thrombin expression.

KRAS, a GTPase signalling protein, regulates cell proliferation, differentiation, and survival and is the human homologue of the Kirsten rat sarcoma-2 viral oncogene[5]. Recent research has linked the KRAS gene to thrombotic lesions, suggesting that it plays a key role in the development of colorectal cancers. KRAS mutations are found in ~30–50% of colorectal tumours and are also common in lung adenocarcinoma and prostate tumours[6–8]. Tumour-mutated KRAS status is associated with an increased risk of venous thromboembolism (VTE) in patients with metastatic colorectal cancer, according to a retrospective multicenter cohort study conducted by Ades[9]. KRAS mutations influence malignancy progression by affecting tissue factor/FVIIa colorectal cancer cells and the EGFR signalling pathway[10]. Compared to KRAS wild-type tumours, KRAS-mutated colorectal cancers have a poorer overall prognosis, a shorter median overall survival, and an increased incidence of deep vein thrombosis[9,10].

[a]Department of General Surgery, Yan'an Hospital Affiliated to Kunming Medical University, Kunming and [b]Key Laboratory of Tumor Immunological Prevention and Treatment of Yunnan Province, Kunming, China

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

*Corresponding author. Address: Department of General Surgery, Yan'an Hospital Affiliated to Kunming Medical University, Kunming 650051, China. Tel.: +18 856 937 041; fax: +861 885 693 7041 E-mail: jingtan0915@163.com (J. Tan).



Annals of Medicine & Surgery (2024) 86:850–855

Received 3 November 2023; Accepted 11 December 2023

Published online 18 December 2023

http://dx.doi.org/10.1097/MS9.0000000000001645

There is growing evidence that platelets play a crucial role in promoting a hypercoagulable state in cancer patients[11]. Tumour cells cause platelet activation through various pathways, including tumour cell-platelet adhesion and the secretion of molecules that activate platelets by tumour cells (e.g. ADP, thrombin, matrix metalloproteinases, and IL-6)[11–13]. However, there is still a lack of KRAS coagulation studies performed at the cellular level in colorectal cancer. We hypothesized that KRAS-mutant cells were more likely to cause a hypercoagulable state in the blood.

We measured platelet aggregation rates after colorectal cancer cell-platelet interactions. The maximum platelet aggregation rate is often used to reflect the status of the incoming thrombus[14]. We used human RKO, HCT116, SW480, and SW620 colorectal cancer cell lines with different KRAS genotypes to explore platelet-colorectal cancer cell interactions. The RKO cells were a wild-type of KRAS, and the other three were mutant cells. Trends in tumour cell-induced platelet aggregation rates were explored by adding the FVIIa/tissue factor inhibitor PCI-27483 and the thrombin inhibitor recombinant hirudin. We further hypothesized that in colorectal cancer cells, the KRAS gene may affect the degree of coagulation by regulating thrombin production and coagulation.

## Material and methods

### Cell culture

We obtained human colorectal cancer cell lines either from our lab, the Chinese Academy of Sciences, or the Shanghai Institute of Biological Sciences. Dulbecco's Modified Eagle's Media (Gibco, Thermo Fisher Scientific, Inc.) was used to treat RKO, SW480, and SW620 cells, whereas McCoy's 5A medium was used to treat HCT116 cells (Nanjing KeyGen Biotech Co., Ltd.). We added 10% foetal bovine serum (Gibco, Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin to the media as supplements. To continue exponential development, subculture was performed every two to three days at 37°C in a humidified environment with 5% $CO_2$ and 95% air.

### Blood samples

We took blood from healthy volunteers who were at least 18 years old for the platelet aggregation test. For the purpose of measuring platelet function, blood was taken with a needle from a peripheral vein and placed into BD Vacutainer tubes (Becton Dickinson). Patients with cancer were excluded. Treatment with platelet-depleting medicines or platelet transfusions within two weeks of sample collection, as well as surgery, invasive vascular manipulation, radiation therapy, or chemotherapy during the previous week of sample collection, also excluded patients from participation. The study involving human subjects was reviewed and approved by the Medical Ethics Committee of Yan'an Hospital, Kunming Medical University, China (Approval No. 2015-049-01). Patients provided written informed consent to participate in this study.

### Platelets interacted with tumour cells

Blood that had been citrate-anticoagulated was centrifuged for 10 min at 200*g* to extract the platelet-rich plasma, which was then put into freezing tubes. We added an acid-citrate-glucose solution and prostaglandin E1 to avoid platelet activation during subsequent centrifugation (Merck KGaA). Centrifugation at 1000*g* for 10 min produced platelet precipitates. The platelet pellet was washed and resuspended in phosphate-buffered saline (PBS). The densities of the four colorectal cancer cell lines in the 24-well plates were comparable. Equal volumes of platelet suspension were added to the cells after they had been washed with PBS and the medium had been discarded. Two additional 24-well plates were similarly prepared and supplemented with the thrombin inhibitor recombinant hirudin ($\geq$ 7000 ATU/mg protein; Merck KGaA) and FVIIa/tissue factor inhibitor PCI-27483 (MedChemExpress). They were incubated in a 37°C 5% $CO_2$ incubator for 1 h. Afterward, all suspensions after the reaction were collected in Eppendorf tubes and labelled to detect platelet aggregation rates. The control group was the platelet suspension that did not react with tumour cells (Fig. 1).

### Determination of the maximum platelet aggregation rate

A PL-12 multiparameter platelet function analyzer (Jiangsu Innovartis Medical Technology Co., LTD.) was used for determination. We used Kurt's principle to judge the function of platelets by directly measuring the change in the number of platelets before and after the addition of the inducer. Arachidonic acid was used as the inducer. This method detected the signal directly, and the result reflected the platelet aggregation.

### Western blotting

Cells were treated by ultrasound on ice in a lysis buffer mixed with a protease and phosphatase inhibitor (Merck KGaA). The lysate was centrifuged at 14 000*g* for 5 min to collect the supernatant. Then, a BCA protein analysis kit quantified the total extracted protein concentration. The loading buffer was added to each lysate and boiled for 5 min. Protein samples were transferred to polyvinylidene difluoride membranes after being resolved by 8% SDS-PAGE (EMD MilliporePrimary antibodies were then incubated on the blots overnight at 4°C after the blots had been blocked with 5% nonfat milk for 1 h at 25°C . Beta-Actin antibody (GB12001, Servicebio) and anti-thrombin antibody (#ab17199, Abcam) were the main antibodies utilized. The secondary antibody, an anti-mouse antibody coupled to horseradish peroxidase (#96356, Cell Signalling Technology), was incubated for 1 h at room temperature. An improved chemiluminescence kit was used to detect the immunoreactive bands on the Tanon4500 Gel Imaging system (Tanon Science and Technology Co., Ltd.).

### Statistical analysis

IBM SPSS Statistics for Windows (Version 22.0, IBM Corp) was used for the statistical analysis. For continuous variables, we utilized the Shapiro-Wilk test for normality and a histogram analysis to determine the distribution of our data. Means and SDs for continuously dispersed, normally distributed data are presented. Single-factor ANOVA (one-way ANOVA) was used to compare several groups' maximum platelet aggregation rates induced by colorectal cancer cells. Multiple post-hoc comparisons were performed using The Dunnett hypothesis covariance with RKO cell lines as control. Paired *t*-tests were used to compare differences in platelet aggregation rates before and after the same cell intervention. A statistically significant difference was defined as $P$ less than 0.05.

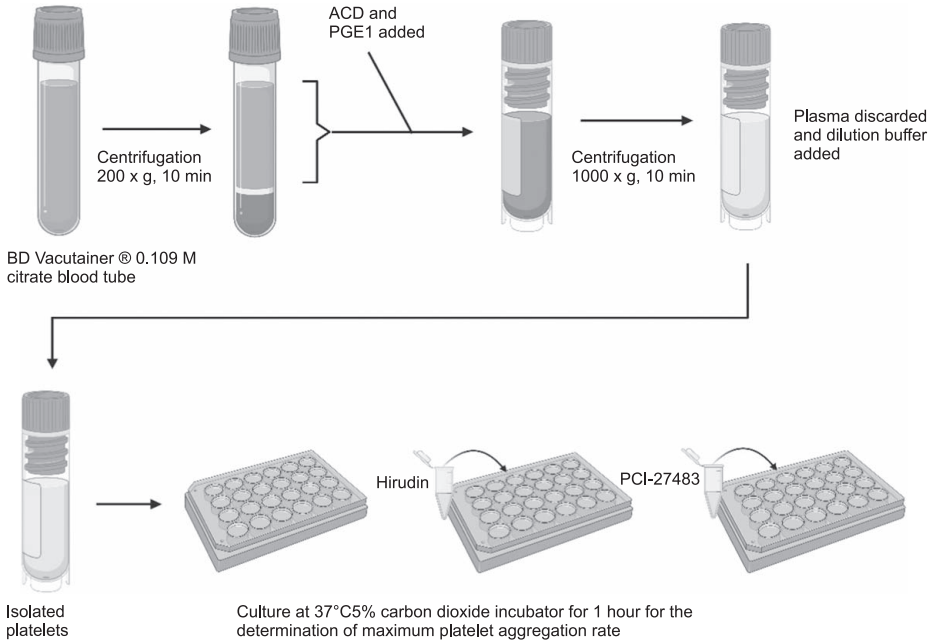


**Figure 1.** Procedures for determining the peak platelet aggregation rate using a platelet function analyzer. ACD, acid-citrate-dextrose; PGE1, prostaglandin E1.

## Results

The platelet aggregation rate was determined by measuring the platelet aggregation rate of each cell tumour line in the hirudin and non-inhibitor groups. The results showed a statistically significant difference in the distribution between the groups in the non-inhibitor group ($P < 0.001$), and in a post-hoc comparison with the RKO cell line as a control, the cell lines HCT116 and SW620 were statistically different (Fig. 2A). In addition, the SW480 cell line derived from in situ rectal adenocarcinoma tissue was statistically different from its metastatic focal SW620 cell line ($P < 0.05$). In the hirudin group, the distribution between the groups was statistically different ($P = 0.001$), and similarly, SW620 was statistically different from RKO in the post-hoc comparison. Results were statistically different ($P < 0.05$) before and after the addition of hirudin in the same cell line or control group (Table 1). When we compared the maximum platelet aggregation rate in the non-inhibitor and the hirudin groups, we found that the three cell lines HCT116, SW480, and SW620 decreased significantly more than RKO after the intervention (Fig. 2B). When comparing the magnitude of their decreases, the difference between RKO and HCT116 before and after the intervention was statistically significant (Difference = 16.80, 95% CI = 2.38–30.82, $P = 0.026$) (Table 2). The difference between RKO and SW620 before and after the intervention was statistically significant (Difference = 14.50, 95% CI = 2.24–26.76, $P = 0.028$) (Table 3). After adding the FVIIa/tissue factor inhibitor PCI-27483, we compared its measured maximum platelet aggregation rate with that of the hirudin group. We found that the inhibition of platelet aggregation induced by tumour cells was similar between the two groups of inhibitors (Fig. 2C). We performed immunoblotting experiments to determine the expression of thrombin in colorectal cancer cell lines to understand the effect of recombinant hirudin on aggregation rates. In contrast to RKO, thrombin expression was shown to be greater in HCT116, SW480, and SW620 cells (Fig. 2D).

## Discussion

In this study, we revealed that at the cellular level, KRAS-mutant colorectal cancer cells were more likely to cause a hypercoagulable state of blood and express higher levels of thrombin compared to wild-type. The association between KRAS mutations and blood hypercoagulability may involve multiple mechanisms. In human colorectal cancer cells, KRAS oncogene activation and p53 tumour suppressor gene inactivation are two major transformation events that mediate tissue factor (TF) overexpression, and their patterns depend on MEK/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3'-kinase (PI3K)[15]. This may be one of the main mechanisms of KRAS leading to blood hypercoagulability.

TF is a coagulation factor VII/VIIa (FVII/VIIa) transmembrane cellular receptor that has a molecular weight of 47 kDa[16,17]. Yu *et al.*[15] demonstrated in a preclinical model that TF upregulation in colorectal cancer cells is directly related to mutation activation of the KRAS oncogene. Rao *et al.*[18] confirmed this in colorectal cancer samples and non-small cell lung cancer[19]. Cancer coagulopathy is usually associated with the upregulation of TF, the initiator of the significant cytokine coagulation cascade[20] and a representative cancer procoagulant[21]. The formation of TF/VIIa complexes on the cell surface triggers the proteolytic conversion of circulating factor X (FX) to the active form (FXa) during the coagulation cascade when TF expression is elevated or the membrane microbubbles containing TF are shed[22]. FXa activates the conversion of thrombinogen (FII) to thrombin (FIIa). The following amplification of thrombin generation is a result of the activity of coagulation factors Va, VIIa, and IX. The subsequent surge of thrombin activity triggers protease-activated

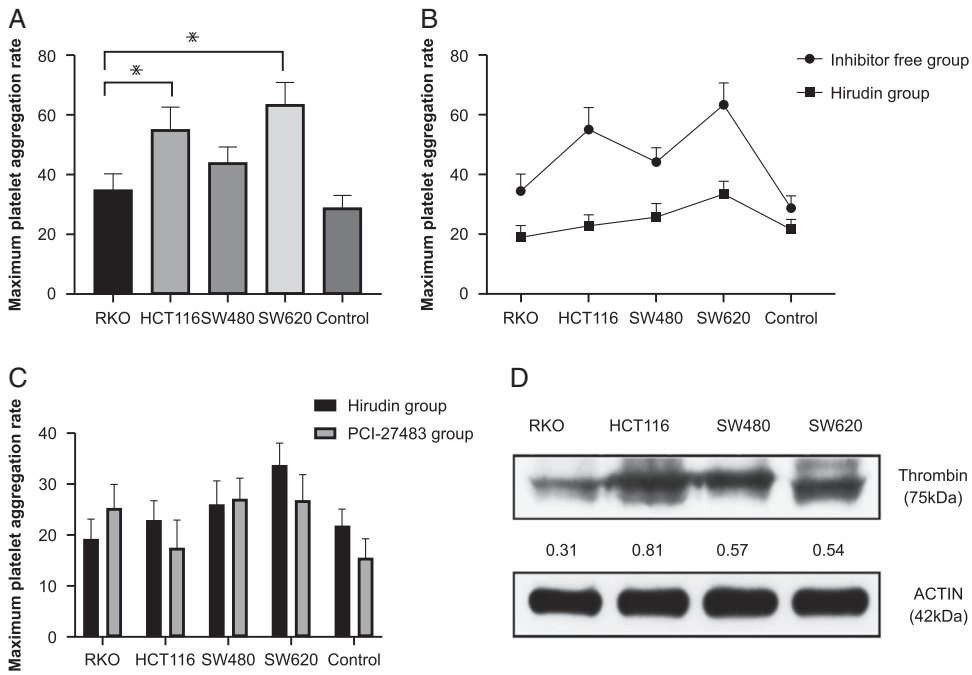


**Figure 2.** The maximum platelet aggregation rate and thrombin expression results differed between KRAS wild-type and mutant cell lines. (A) The maximum platelet aggregation rate in the inhibitor-free group was higher and statistically different in the HCT116 and SW620 cell lines compared to the RKO cell line (*$P < 0.05$). (B) Trends in maximum platelet aggregation rates for each cell line and control group before and after the addition of inhibitors. (C) Comparison of the maximum platelet aggregation rate after the addition of the FVIIa/tissue factor inhibitor PCI-27483 and the thrombin inhibitor hirudin. (D) The expression of thrombin was higher in HCT116, SW480, and SW620 cell lines than in RKO cell lines.

receptors involved in platelet activation and converts soluble fibrinogen to insoluble fibrin, both of which are necessary for fast clotting[23]. In conclusion, our study suggests that KRAS mutations in colorectal cancer cells are more likely to lead to a hypercoagulable state of blood, consistent with the results of Ades' cohort study, which showed a higher incidence of venous thromboembolism in KRAS-mutant patients. Although KRAS mutations do not necessarily cause VTE, the hypercoagulable state increases the risk of VTE and correlates with tumour progression and metastasis.

Furthermore, preclinical evidence implies that TF participates in a number of cancer-related processes, including metastasis, angiogenesis, and tumour development[24–27]; the same has been reported in colorectal cancer[28,29]. Furthermore, increased TF expression was associated with a worse outcome in colorectal cancer patients[18]. These results are in line with the rises in TF expression seen in various colorectal cancer clinical stages at Duke[29]. TF expression in tumour cells is a crucial link between cancer and cancer-related thrombosis[30]. In addition, cancer-related thrombosis can lead to a poor prognosis for tumour patients. We speculated that the KRAS status may become essential in evaluating the risk of blood hypercoagulability in colorectal cancer patients and an indicator of poor prognosis.

In our experiments, we found a statistically significant decrease in the maximum platelet aggregation rate following the addition of recombinant hirudin, suggesting that thrombin plays a vital role in the platelet aggregation response induced by colorectal cancer cells. The slope of the platelet aggregation rate appeared to be flatter in the hirudin group than in the non-inhibitor group, with the decrease in HCT116 and SW620 being statistically different from that of RKO. This is broadly similar to the expression of thrombin in colorectal cancer cells.

Furthermore, the higher rate of maximum platelet aggregation induced by the metastatic tumour strain SW620 compared to the

**Table 1**

**Determination of the maximum platelet aggregation rate of colorectal cancer cell lines after platelet interaction before and after the addition of recombinant hirudin.**

| | | | Paired *t*-test | |
|---|---|---|---|---|
| Groups | Inhibitor-free group (Mean ± SD) | Hirudin group (Mean ± SD) | *t* | *P* |
| RKO | 34.73 ± 5.68 | 19.10 ± 3.99 | 5.21 | 0.014 |
| HCT116 | 55.40 ± 7.48[a] | 22.98 ± 3.74 | 6.65 | 0.007 |
| SW480 | 44.43 ± 4.86 | 25.85 ± 4.72 | 5.55 | 0.012 |
| SW620 | 63.80 ± 7.29[a] | 33.68 ± 4.34[a] | 7.50 | 0.005 |
| Control | 28.98 ± 4.04 | 21.80 ± 3.29 | 4.67 | 0.019 |
| F-value | 22.71 | 7.614 | | |
| *P* value | < 0.001 | 0.001 | | |

[a]Represents the comparison with RKO; $P < 0.05$.

**Table 2**

**Comparison of the difference between RKO and HCT116 before and after the addition of recombinant hirudin.**

| Group | Mean ± SD | Difference value (95% CI) | *P* |
|---|---|---|---|
| RKO | 15.62 ± 6.00 | 16.80 (2.38–30.82) | 0.026 |
| HCT116 | 32.43 ± 9.76 | | |

**Table 3**

**Comparison of the difference between RKO and SW620 before and after the addition of recombinant hirudin.**

| Group | Mean ± SD | Difference value (95% CI) | *P* |
|---|---|---|---|
| RKO | 15.62 ± 6.00 | 14.50 (2.24–26.76) | 0.028 |
| SW620 | 30.13 ± 8.03 | | |

primary rectal cancer tumour strain SW480 may correlate with the degree of gene expression and is consistent with relevant clinical reports of higher incidence of thrombotic adverse events in patients with colorectal cancer with metastasis[3]. In a comparison between the two inhibitors, PCI-27483 and hirudin, we found that the maximum platelet aggregation rate decreased to a similar extent in both groups, suggesting that platelet aggregation induced by colorectal cancer tumour lines is mainly achieved through the TF-thrombin pathway. Two critical mediators in the link between coagulation and malignancy have been suggested by Ricicles *et al*.[26], namely thrombin and TF. The clotting-dependent pathway involves activation of the TF receptor via ligand binding and subsequent downstream production of thrombin and clot formation[31,32]. We hypothesized that KRAS gene mutation-mediated TF upregulation and subsequent increased thrombin expression in colorectal cancer cells is a primary pathway leading to a hypercoagulable state of the blood.

Colorectal cancer cells can contribute to a hypercoagulable state of blood through various mechanisms, including tumour procoagulant, which is not present in any normal tissue and can activate coagulation factor X independently of coagulation factor VIII[33]. It has also been reported that p53 and BRAF genes are associated with a hypercoagulable state of blood[34] and that PTEN mutations are associated with increased TF expression, which causes an increase in thrombin expression[25]. Indeed, many oncogenes or tumour suppressor genes and their mutations affect the up- or downregulation of coagulation-associated substance production by tumour cells. This may be why the maximum platelet aggregation rate of the KRAS-mutant cell line SW480 was insignificant in this experiment compared to RKO.

The limitation of this study is that it only explored the effect of a single gene, KRAS, in colorectal cancer cells on coagulation function. There is a need for reproducible experimental studies to compare the differences in the procoagulant effects of different genotypes of cancer cells, which can help us gradually reveal the relationship between mutations in nodal cancer genes and hypercoagulable states and provide more precise anticoagulation and antitumor treatment options for tumour patients. The coagulation response induced by TF in colorectal cancer cells needs further study, including its effects on KRAS and thrombin.

## Conclusion

Our experiments show that KRAS-mutated colorectal cancer cells are more capable of inducing a thrombotic state. This manifestation may be induced by the expression of thrombin in tumour cells and is closely linked to tissue factor.

## Ethical approval

The study involving human subjects was reviewed and approved by the Medical Ethics Committee of Yan'an Hospital, Kunming Medical University, China (Approval No. 2015-049-01). Patients provided written informed consent to participate in this study.

## Consent

Not applicable.

## Sources of funding

This study was supported by grants from the National Natural Science Foundation of China (No. 81960499).

## Author contribution

D.X. made substantial contributions to the concept and design. C.L. made substantial contributions to the acquisition of data and the analysis and interpretation of the data. J.T. were involved in drafting the article or revising it critically for important intellectual content. All authors read and approved the final paper.

## Conflicts of interest disclosure

The authors declare no conflicts of interest.

## Research registration unique identifying number (UIN)

1. 1.Name of the registry:
2. 2.Unique Identifying number or registration ID:
3. 3.Hyperlink to your specific registration (must be publicly accessible and will be checked):

## Guarantor

Duogang Xu is the person in charge of the publication of our manuscript.

## Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Provenance and peer review

Not commissioned, externally peer-eviewed.

## References

[1] Sinicrope FA. Increasing incidence of early-onset colorectal cancer. N Engl J Med 2022;386:1547–58.
[2] Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. Blood 2007;110:1723–9.
[3] Horsted F, West J, Grainge MJ. Risk of venous thromboembolism in patients with cancer: a systematic review and meta-analysis. PLOS Med 2012;9:e1001275.
[4] Kawai K, Watanabe T. Colorectal cancer and hypercoagulability. Surg Today 2014;44:797–803.
[5] Jackson EL, Willis N, Mercer K, *et al*. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev 2001;15:3243–8.

[6] Andreyev HJ, Norman AR, Cunningham D, *et al*. Kirsten ras mutations in patients with colorectal cancer: the "RASCAL II" study. Br J Cancer 2001;85:692–6.
[7] Di Fiore F, Blanchard F, Charbonnier F, *et al*. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 2007;96:1166–9.
[8] Lièvre A, Bachet JB, Le Corre D, *et al*. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006; 66:3992–5.
[9] Ades S, Kumar S, Alam M, *et al*. Tumor oncogene (KRAS) status and risk of venous thrombosis in patients with metastatic colorectal cancer. J Thromb Haemost 2015;13:998–1003.
[10] Chen HK, Wang X, Wan YL, *et al*. Crosstalk between TF/FVIIa and EGFR signaling in colorectal cancer cells. Cancer Biol Ther 2019;20:454–60.
[11] Cremolini C, Loupakis F, Antoniotti C, *et al*. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. Lancet Oncol 2015;16:1306–15.
[12] Connolly GC, Phipps RP, Francis CW. Platelets and cancer-associated thrombosis. Semin Oncol 2014;41:302–10.
[13] Falanga A, Russo L, Verzeroli C. Mechanisms of thrombosis in cancer. Thromb Res 2013;131 Suppl 1:S59–62.
[14] Lee EC, Cameron SJ. Cancer and thrombotic risk: the platelet paradigm. Front Cardiovasc Med 2017;4:67.
[15] Yu JL, May L, Lhotak V, *et al*. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. Blood 2005;105:1734–41.
[16] Mackman N, Morrissey JH, Fowler B, *et al*. Complete sequence of the human tissue factor gene, a highly regulated cellular receptor that initiates the coagulation protease cascade. Biochemistry 1989;28:1755–62.
[17] Chen J, Bierhaus A, Schiekofer S, *et al*. Tissue factor--a receptor involved in the control of cellular properties, including angiogenesis. Thromb Haemost 2001;86:334–45.
[18] Rao B, Gao Y, Huang J, *et al*. Mutations of p53 and K-ras correlate TF expression in human colorectal carcinomas: TF downregulation as a marker of poor prognosis. Int J Colorectal Dis 2011;26:593–601.
[19] Regina S, Rollin J, Bléchet C, *et al*. Tissue factor expression in non-small cell lung cancer: relationship with vascular endothelial growth factor expression, microvascular density, and K-ras mutation. J Thorac Oncol 2008;3:689–97.
[20] Kakkar AK, DeRuvo N, Chinswangwatanakul V, *et al*. Extrinsic-pathway activation in cancer with high factor VIIa and tissue factor. Lancet 1995;346:1004–5.
[21] Falanga A. Thrombophilia in cancer. Semin Thromb Hemost 2005;31: 104–10.
[22] Giesen PL, Rauch U, Bohrmann B, *et al*. Blood-borne tissue factor: another view of thrombosis. Proc Natl Acad Sci USA 1999;96:2311–5.
[23] Coughlin SR. Protease-activated receptors in vascular biology. Thromb Haemost 2001;86:298–307.
[24] Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. Arterioscler Thromb Vasc Biol 2004;24:1015–22.
[25] Rak J, Milsom C, May L, *et al*. Tissue factor in cancer and angiogenesis: the molecular link between genetic tumor progression, tumor neovascularization, and cancer coagulopathy. Semin Thromb Hemost 2006;32: 54–70.
[26] Rickles FR, Patierno S, Fernandez PM. Tissue factor, thrombin, and cancer. Chest 2003;124(3 Suppl):58S–68S.
[27] Khorana AA, Ahrendt SA, Ryan CK, *et al*. Tissue factor expression, angiogenesis, and thrombosis in pancreatic cancer. Clin Cancer Res 2007;13:2870–5.
[28] Nakasaki T, Wada H, Shigemori C, *et al*. Expression of tissue factor and vascular endothelial growth factor is associated with angiogenesis in colorectal cancer. Am J Hematol 2002;69:247–54.
[29] Seto S, Onodera H, Kaido T, *et al*. Tissue factor expression in human colorectal carcinoma: correlation with hepatic metastasis and impact on prognosis. Cancer 2000;88:295–301.
[30] van den Berg YW, Osanto S, Reitsma PH, *et al*. The relationship between tissue factor and cancer progression: insights from bench and bedside. Blood 2012;119:924–32.
[31] Rickles FR, Shoji M, Abe K. The role of the hemostatic system in tumor growth, metastasis, and angiogenesis: tissue factor is a bifunctional molecule capable of inducing both fibrin deposition and angiogenesis in cancer. Int J Hematol 2001;73:145–50.
[32] Fernandez PM, Rickles FR. Tissue factor and angiogenesis in cancer. Curr Opin Hematol 2002;9:401–6.
[33] Falanga A, Consonni R, Marchetti M, *et al*. Cancer procoagulant and tissue factor are differently modulated by all-trans-retinoic acid in acute promyelocytic leukemia cells. Blood 1998;92:143–51.
[34] Ortega Morán L, García Alfonso P, Aguilar Caballero I, *et al*. Incidence of venous thromboembolism in patients with colorectal cancer according to oncogenic status. Clin Transl Oncol 2020;22:2026–31.