The Clinical Detection and Significance of DNA Ploidy in Nucleus of Rectal Cancer Cell

Mingliang Lu[1], Linfu Lin[2]

[1] Department of Anus Intestines, PLA 117 Central Hospital (Hangzhou 310013)
[2] Department of General Surgery, Changhai Hospital (Shanghai 200433)

Abstract Objective to study the relationship between DNA content of nucleus of rectal cancer cell and clinical sign of rectal cancer and find the mechanism of DNA ploidy's role in tumor genesis and development through the relationship between DNA ploidy and pathological sign so as to provide basis for preoperative tumor complex treatment and resection range. **Methods** DNA content was studieel in 87 patieats of rectal cancer with filuores cance activated cell assay instrument (FCA star model) **Result** The increase of DNA heteroploid was highly associated with rectal cancer quick genesis, metastasis and differentiation. Along with the metastasis, deeper or larger extent invasion of rectal cancer, the incidence and content of DNA heteroploid increased strikingly. Heteroploid aggregated mainly in tumor Dukes B and C stage while posterior stage incidence was higher than anterior stage. The 5-year survival rate of patients with DNA heteroploid was significantly lower than DNA diploid ($p<0.05$). Moreover, the re-radical operation opportunity of patients with DNA heteroploid will significantly decrease when cancer presents recurrence or metastasis after rectal cancer radical operation. **Conclusion** The content of DNA ploidy in rectal cancer patient can be an availability index of preoperative complex treatment, resection range ascertainment and prognosis estimation.

Key Words rectal carcinoma; DNA ploidy; clinical significance

The study of DNA content and ploidy in rectal cancer is of great significance to the understanding of biological behavior of rectal cancer cells, clinical treatment guidance and prognosis estimation. Meanwhile, the variety of DNA content plays an important role in carcinoma genesis, development, metastasis and recurrence [1]. The relationship between DNA content and clinical pathology was studied in 87 patients in this study. The result indicates that DNA heteroploid has a close relation to the alteration of carcinoma biological behavior[2].

MATERIALS AND METHODS

Clinical data

All the paraffin embedding samples were chosen from 87 patients who underwent radical operation of rectal cancer in our hospital from 1996 to 1997. We chose the patients whose conditions were as the following: 1. Primary rectal cancer case. 2. No radiotherapy and chemotherapy before operation. 3. Performed radical operation of rectal cancer and was confirmed by pathological section. The ages of all the patients (male 56, female 31) were from 26 to 73 (average 54.1). The most symptoms were stool habit alteration, bloody purulent stool, stool deformation as thinner or impression. 61 cases felt uncomfortable because of anal descensus while 18 cases had rectal incomplete block symptom. Anal indications: The tumor is located at 2 to 5cm from anal edge in 42 cases, at 5 to 8cm from anal edge in 33 cases, at 8 to 12cm (can not touch by digital rectal examination) in 12 cases. 56 cases were prominence-bump type tumor and 31 were ulcer-infiltration type tumor. 47 cases were canalicular adenoma of rectal, 25 were mucinous adenocarcinoma, 12 were papillary adenocarcinoma and 3 had signet-ring cell carcinoma.

Preparation of cells suspension

3 to 5 pieces of 40μ m thick tissue section were prepared from paraffin embedding tissue and laid in test tube. 3 to 5ml xylol was added into tube and stored at room temperature for 48h. 100%, 95%, 70% and 50% gradient alcohol was added in turn to hydrate at the interval of 10 minutes. Distilled water (3 to 5 ml) was added and abandoned after 5 minutes. Then 5ml 0.5% pepsin was added and digested in 37℃ constant-temperature water for 30 minutes. The digestion was stopped by 3 to 5ml normal saline. Cells suspension was collected by 300-well nylon filtration and centrifugated at 1500prm. Before the second centrifugation the cells were rinsed by normal saline 1 or 2 times. 70% alcohol was used to fixed cells after the supernatant was abandoned and stored in 4℃ refrigeratory. Paraffin section from normal intestine proximal to carcinoma was used for inner comparison.

2. FCM detection

After centrifugation and abandonment of fixative solution from samples, normal cells were added as inner standard and ethidium bromide fluorescence dying was performed. Fluorescence activated cell assay instrument (FCA star model, produced by USA Becton Dickinson company) was used. Fluorescence signals from cell DNA were registered in multipulse assay implement, showed on screen with histogram and datasheet manner and then printed out.

3. Result determinant

Carcinoma cell ploidy was determined by Friedlander criterion, that is, diploid cells had only one G1 peak value. If there was additional G1 peak on the histogram, we consider it the cell heteroploid. The DNA content was recorded as either diploid or heteroploid.

4. Survival rate investigation

Each patient had follow-up survey of 1 to 5 years and complete reexamination include CEA, SF, ultrasonic examination by Doppler's method of abdominal and pelvic cavity, X-ray of chest and electronic enteroscope per 4-6 months. All the patients had integrated survey record and should be done more reexaminations in order to get a definitive diagnosis when suspected node was found.

RESULTS

DNA content detection

All the patient DNA content detections were successful. 32 cases were diploid DNA (36.8%) while 55 cases were heteroploid (63.2%).

The relationship between pathological characteris–tics and DNA polidy

Pathologic changes range Length of the (lesion) tumor pathologic changes was from 2 to 12cm (average 5cm). 20 cases (55.6%) were DNA heteroploid, and 16 cases were diploid in all the 36 cases whose carcinoma invaded less than 1/2 circumference of rectum. 35 cases (68.6%) were DNA heteroploid, and 16 cases (invaded whole circumference) were diploid in all the 51 cases whose carcinoma invaded more than 1/2 circumference of rectum.

Tumor penetration depth 4 cases invaded in superficial muscle (DNA diploid 2, heteroploid 2), 15 cases were invaded in deep muscle layer (diploid 4, heteroploid 11), 29 cases were invaded in serous coat (diploid 10, heteroploid 19) and 39 cases were invaded out of serous coat (diploid 16, heteroploid 23). Among all layers, DNA ploidy had no statistical significance ($p>0.05$).

Pathological classification 16 cases were well-differentiated carcinoma (6 heteroploid, 37.5%); 51 cases were middle-differentiated carcinoma (32 heteroploid, 63%); 20 cases were poor-differentiated carcinoma (17 heteroploid, 85%). The incidence of DNA heteroploid in poor-differentiated carcinoma was significantly higher than in well-differentiated or middle-differentiated carcinoma.

Invasion of adjacant tissue 21 cases had around tissue invaded. Most of the tissue and organs were prostate, bladder, posterior wall of vagina and fascia in front of sacrum. Among them the ureter was invaded in 3 cases (1 case has the common iliac artery invaded at the same time). In this group, DNA content detection shown 3 cases of diploid, 18 cases of heteroploid. Among the 66 cases that adjacent tissue and organs had not been obviously invaded, 29 were diploid and 37 were heteroploid.

Dukes stage relation with DNA content 5 cases with Dukes A stage had no DNA heteroploid; 41 cases with Dukes B stage had 17 cases of diploid and 24 cases of heteroploid among them; 32 cases with Dukes C stage had 9 of diploid and 23 of heteroploid; 9 cases with dukes D stage had 1 of diploid and 8 of heteroploid. DNA heteroploid cases concentrated on B and following stage mainly. The DNA heteroploid incidence of B stage was 58.5%, C was 71.9%, and D was up to 88.9%.

Lymph node metastasis 26 cases have lymph node metastasis while 61 had no lymphatic metastasis, the metastasis rate of lymph node was 29.9%. The content of heteroploid accounted for 88.5% in lymphatic metastasis cases but only 52.5% in no lymphatic metastasis cases. Statistical assay indicates that there is remarkably difference of DNA contents between lymphatic metastasis group and no metastasis group ($p< 0.05$).

The relationship between survival rate and DNA polidy

We made a follow-up survey on this group and found 7 patients had tumor metastasis (recurrence) 1 year after the operation. Among them DNA diploid was in one patient whose recurrent tumor was totally exercised by re-operation. And DNA heteroploid was in 6, a-

Table 1. The relationship between lymphatic metastasis and DNA content

Lymphnode metastasis	DNA content		Cases
	Diploid	Heteroploid	
Yes	3	23	26
No	29	32	61
Total	32	55	87

$X^2=4.835$ $p<0.05$

Table 2. The relationship of content of rectal cancer cell DNA and 5-year survival rate

Life cycle	DNA content		Total
	Diploid	Heteroploid	
⩾5 years	29	2	50
<5 years	3	34	37
Total	32	55	87
5-year survival rate	90.6%	38.2%	57.5%

$X^2=6.429$ $p<0.05$

mong them 3 had already presented widespread metastasis at the time of going to a doctor and no chance of operation, the other 3 cases had operations again (2 was basic radical operation, 1 was palliative operation). 21 patients had tumor metastasis in 3 years, among them 7 were diploid and 14 were heteroploid. 5-year survivors were 50 cases. Carrying-tumor survivors were 12, and among them were 4 cases of diploid (3 once had twice radical operations) and 8 cases of heteroploid (3 had twice operations and 1 had three times excision). In this group 37 patients lived less than 5 years after operation, among them 11 less than 3 years (3 less than 1 year), while 50 cases up to more than 5 years (5-year survival rate is 57.5%). There is obvious relationship between the rectal cancer cell DNA ploidy and, 3-year survival rate, 5-year survival rate after operation and tumor recurrence. And re-excision rate is obviously different between different ploidy after recurrence. This group shows that tumor recurrence rate increases obviously and 5-year survival rate decreases with the increase of rectal cancer cell DNA ploidy. The difference between two types of ploidy is remarkable ($p<0.05$). The relationship between DNA content and 5-year survival rate after operation is shown in Table 2.

DISCUSSION

Appraisal of rectal carcinoma growth way, malignant degree and prognosis depended mainly on pathological characteristics such as tumor stages and histology grading in the past. In recent years, with the rapid development of molecular genetics and application of the automatic cell's detection technique, the view that DNA content is closely related with the biological behavior in the tumor cell was put forward. Tumor cell presents DNA content increasing often because of nucleic acid metabolic abnormity or variation of chromosome. Tumor presents high DNA content and more heteroploid cell should show obvious malignant degree and poor prognosis[3].

Local infiltration, lymphatic metastasis of rectal carcinoma had close relations with DNA heterogeneity. In this study, DNA heteroploid increases obviously with rectal cancer cell's differentiation degree decreasing, and it is significantly different from diploid content. The rectal cancer with DNA heteroploid has stronger ability to infiltrate surrounding tissue than that with DNA diploid. There is between remarkable difference of DNA content ($p<0.05$) lymphatic metastasis, and non-me-tastasis, which indicates heteroploid rectal carcinoma is easier to develop lymphatic metastasis than diploid carcinoma. This is the same as previous reports [1,4,5,6]. This study shows traditional pathomorphology grading can differentiate obvious paramorphia tumors more accurately but differentiate the tumors which has similar morphosis but different biological behavior difficultly. So combining DNA content determination with traditional pathology grading should be beneficial to judging tumor malignant

degree.

Research has shown rectal carcinoma cell DNA heteroploid appeared mainly on Dukes B and C stage. Diploid and heteroploid appear in Dukes C higher than Dukes B obviously. With the tumor metastasis and infiltration, the incidence of cell DNA heteroploid increases significantly[7,8,9,10,11]. This indicates that DNA ploidy style and chromosome conformation of rectal cancer cell are closely related with its growth, infiltration and lymphatic metastasis. Shigemichi, et al[4]. measured 148 DNA content of rectal carcinoma with FCM and found that 21.6% of high heteroploid (HAP) content tumor occurred distance metastasis, but the distance metastasisin low heteroploid (LAP) content and diploid (DP) were 6.4% and 2.8% respectively after operation, there is statistical significance between them (p<0.05). We made the follow-up survey of this group and found 5-year survival rate of diploid and heteroploid case was 90.6% and 38.2% respectively (statistical significance, p<0.05). This indicates that with the increase of DNA content and abnormal ploidy degree of carcinoma, the prognosis is getting worse. Consequently the detection of content of rectal carcinoma cell DNA ploidy should be an important index of prognosis appraisal.

REFEREBCES

1. Geido E, Sciutto A, Rubagotti A, et al. Combined DNA flow cytometry and sorting with k-ras2 mutation spectrum analysis and the prognosis of human sporadic colorectal cancer. Cytometry, 2002, 50(4): 216-224.
2. Berczi C, Bocsi J, Bartha I, et al. Prognostic value of DNA ploidy status in patients with rectal cancer. Anticancer Res. 2002, 22(6B):3737-3741.
3. Sugai T, Takahashi H, Habano W, et al. Analysis of genetic alterations, classified according to their DNA ploidy pattern, in the progression of colorectal adenomas and early colorectal carcinomas. J Pathol, 2003, 200(2):168-176.
4. Zou L, Mao Y, Lei S, et al. The relationship of cellular DNA content with clinical stage and biological features of colorectal cancer. Hua Xi Yi Ke Da Xue Xue Bao, 2000, 31(2):180-182.
5. Bazan V, Migliavacca M, Zanna I, et al, DNA ploidy and S-phase fraction, but not p53 or NM23-H1 expression, predict outcome in colorectal cancer patients. Result of a 5-year prospective study. J Cancer Res Clin Oncol, 2002, 128(12): 650-658.
6. Russo A, Migliavacca M, Zanna I, et al, p53 mutations in L3-loop zinc-binding domain, DNA-ploidy, and S phase fraction are independent prognostic indicators in colorectal cancer: a prospective study with a five-year follow-up. Cancer Epidemiol Biomarkers Prev, 2002, 11(11):1322-1331.
7. Giaretti W, Venesio T, Sciutto A, et al. Near-diploid and near-triploid human sporadic colorectal adenocarcinomas differ for KRAS2 and TP53 mutational status. Genes Chromosomes Cancer, 2003, 37(2):207-213.
8. Molnar B, Bocsi J, Karman J, et al, Immediate DNA ploidy analysis of gastrointestinal biopsies taken by endoscopy using a mechanical dissociation device.Anticancer Res. 2003, 23(1B): 655-660.
9. Sugai T, Habano W, Uesugi N, et al. Molecular validation of the modified Vienna classification of colorectal tumors. J Mol Diagn, 2002, 4(4):191-200.
10. Fernebro E, Halvarsson B, Baldetorp B, et al. Predominance of CIN versus MSI in the development of rectal cancer at young age. BMC Cancer, 2002, 2(1):25.
11. Chen HS, Sheen-Chen SM, Lu CC. et al. DNA index and S-phase fraction in curative resection of colorectal adenocarcinoma: analysis of prognosis and current trends. World J Surg, 2002, 26(5):626-630.