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THE PROGNOSTIC VALUE OF CYTOGENETIC MARKERS FOR EARLY DIAGNOSIS OF COLORECTAL CANCER

*M. Lozynska**
Institute of Hereditary Pathology AMC of Ukraine, Lviv 79000, Ukraine

Aim: **To investigate the spectrum of chromosome changes in colorectal adenomas and adenocarcinomas and to evaluate the prognostic significance of the chromosome rearrangements.** ***Methods:*** **The study was carried out using the cytogenetic analysis of biopsy specimens (n = 56) of single and multiply adenomas (familial adenomatous syndromes; n = 38) and adenocarcinomas (n = 18).** ***Results:*** **The karyotype of adenomas was normal in the majority of cases, but some adenomas with severe dysplasia of epithelium carry the quantitative chromosome abnormalities and structural rearrangements. The combination of additional copies of chromosomes 13, 18, 20 in adenomas points on an unfavorable prognosis. The chromosome abnormalities were found in 100% of adenocarcinomas biopsy specimens.** ***Conclusions:*** **The transition from colorectal adenomas to adenocarcinomas is accompanied by elevation of chromosome abnormalities level, in particular, by increased clonal variety, selective accumulation of the copies of chromosomes 2, 3, 20, 16, with simultaneous monosomy of chromosomes 17, 18, 8, 6, 14, and del 1p.**
Key Words: **karyotype, chromosome abnormalities, colorectal adenoma, adenocarcinoma.**

Genetics disorders a key role in predisposition to colorectal cancer (CRC) in its initiation and progression. CRC provides a useful model for understanding cancer genetics because CRC development is a multistep process in the majority of cases [1], based on the mutations in several genes. The discovery of genes, involved in carcinogenesis and associated with CRC, including hereditary cancer (*p53, K-ras, MLH1, MSH2*, etc.), play an important role in the creation of the specific highly sensitive tests [2, 3]. Another genetic test is based on determination of DNA quantity in tumor cells using cytospectrophotometric analysis, due to significant role of the changes in DNA content (ploidy level) during the clinical course of CRC adenocarcinomas. This method allows to reveal domination of the polyploid cells in colorectal tumors and correlation of polyploidy with histologically confirmed carcinomas [4]. However, it is not suitable for the evaluation of the spectrum of cytogenetic changes and their prognostic significance.

There is an evident linkage between the appearance of structural and functional alterations in the chromosomes of tumor cells and the origin of CRC. The results of cytogenetic and molecular genetic methods confirm the consequent development of impairments in large bowel (perhaps, except hereditary non-polyposis CRC — Lynch syndrome) [3, 5, 6]. It means that each step of malignancy may accompany chromosomal changes. It is considered that in the majority CRC adenocarcinomas originate on the basis of polyps. Unlike single adenomatous polyps (as a rule histologically benign), multiple polyps (such as familial adenomatous polyps — FAP), possess extremely high malignancy index. Therefore, the morphological structure is the same in adenomas, both single (non-hereditary form of the disease) and multiply (hereditary form). Nevertheless, the frequency of malignancy in both cases is different. Literature data are rather controversial regarding the spectrum and the prognostic value of the chromosome rearrangements during development of CRC [7–12], and the search for informative genetic criteria for early diagnosis of CRC is required.

The aim of this work is to compare the spectrum of chromosome changes in colorectal adenomas and adenocarcinomas, and to evaluate the prognostic significance of chromosome rearrangements in CRC.

MATERIALS AND METHODS

Patients (n = 56) with clinically, endoscopically and histologically confirmed diagnosis of colorectal adenomas and adenocarcinomas were cured in the Department of Surgical Diseases of Danylo Halytsky Lviv National Medical Universiti, Lviv, Ukraine in 1998–2008. All studies were performed according to the Institution's Ethical Committee regulations, and written consents from patients were obtained.

The hereditary nature of the diseases was confirmed using genealogical analysis. Colorectal adenomas (n = 38) were classified as tubular adenomas of single polyps (n = 9), tubulovillous adenomas (n = 10), villous adenomas (n = 10), and tubular adenomas (FAP) (n = 9). All patients with sporadic adenomas were 33 to 86 years old (median age = 64.2 years), and patients with FAP were 22 to 56 years old (median age = 40.8 years).

Patients with adenocarcinoma of large bowel (n = 18) were from 45 to 76 years old (median age = 61.7 years). CRC tumor progression was estimated using the TNM classification. Chromosome abnormalities were described according to the standard International System for Human Cytogenetic Nomenclature [13].

Preparation of the biopsy material of adenomas specimens for cytogenetic analysis was performed according to a standard technique [8] with own modification of cell fixation technique. The metaphase chromosomes of adenocarcinomas specimens were obtained by our own method [14].

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*Correspondence: E-mail: maria_lozynska@ukr.net
Abbreviations used: CRC – colorectal cancer; FAP – familial adenomatous polyps; HNPCC – hereditary non-polyposis colorectal cancer.

The specimens of tumors were mechanically disrupted, washed in a Hanks solution. Then the material was placed in flask with Igle medium with double set of aminoacids, 0.5 μg/ml colchicine solution and ethidium bromide. Then the cells were placed in a mixture of 0.075 M KCl and 1% sodium citrate, fixed in ethanol and acetic acid, and macerated or dropped. The specimens were differentially stained with the Romanovskyy — Gimsa stain dissolved in the phosphate buffer (pH 6.8) supplemented with 0.25% tripsin solution. 5–23 metaphase plates per specimen were analyzed.

RESULTS AND DISCUSSION

The karyotype peculiarities (the spectrum of chromosome anomalies and the sets with alterated ploidy) were studied in morphologically different benign tumors of large bowel. Aneuploidy prevailed in the spectrum of chromosome anomalies in single adenomas. The additional copies of chromosomes 7, 13, 18, 20 and their combinations (7 and 13; 7 and 20, 13 and 20 and al.) were the most frequent abnormalities. Eleven (37.9%) sporadic adenomas and two adenomas with infiltrating carcinoma had abnormal karyotypes. The karyotype was normal in remaining cases. The abnormal karyotype in tubular adenomas was found in 3 from 9 (33.3%) cases. Chromosome abnormalities appeared more frequently in tubulovillous and in villous adenomas: 4 from 10 (40%) and 6 out of (60.0%) cases, respectively. The spectrum of chromosome abnormalities and the degree of epithelial dysplasia in the adenomas are shown in Table 1.

The adenoma in sigmoid colon was observed in one case on the height of 35 cm from the anus, located on the distance of 25 cm lower than high differentiated adenocarcinoma in 57-years old patient (case 13, Table 1). This adenoma with severe dysplasia had 0.7 x 0.5 cm dimensions. The combination of additional copies of chromosomes 13, 18 and 20 was found in the karyotype of this adenoma (Fig. 1).

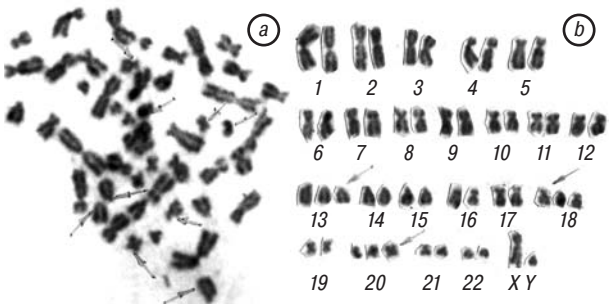

Fig. 1. Karyotype: 48,XY,+13,+18,+20. Villous adenomas of sigmoid colon: *a*, metaphase plate; *b*, ideogram

In the cases of *in situ* adenocarcinoma the metaphase plates contained chromosomal sets with alterated ploidy with modal quantity from 78 to 130 chromosomes (case 7, Table 1), fragment of chromosome that developed as a result of chromosome deletion, and additional copies of chromosomes 7 and 20 (case 8, Table 1). Some authors report quantitative changes of several chromosomes (2, 7, 14) in cases of these intermediate conditions (Table 2) [8, 9].

Table 1. The karyotypes of sporadic and familial adenomas with different degree of epithelial dysplasia

Types of adenomas	Adenomas localization	N of case	Karyotype	The degree of epithelial dysplasia
			Sporadic form	
Tubular adenomas	Sigmoid colon	1	47,XX,+7[8]*;	Severe
	Sigmoid colon	2	47,XY,+7[2]*/48,XY,+7,+13[6]*;	Severe
	Sigmoid colon	3	47,XX,+7[3]*/48,XX,+7,+13[3]*	Severe
Tubular-villous adenomas	Descending colon	4	49,XY,+13,+20,+20[2]*/48,XY,+13,+20[7]*;	Severe
	Sigmoid colon	5	48,XY,+13,+20[3]*/49,XY,+13,+18,+20[6]*;	Severe
	Sigmoid colon	6	52,XY,+7,+del(8q),+13,+14,+15,+20[3]*/50,XY,+7,+13,+15,+20[8]*;	Severe
	Sigmoid colon	7	78,XXX[4]*/82,XXXX[1]/130,XXXXX[1]*	Severe with areas of carcinoma *in situ*
Villous adenomas	Rectum	8	48,XY,+7,+20[3]*/49,XY,+7,+20,+mar[5]*;	Severe with areas of carcinoma *in situ*
	Rectum	9	49,XY,+7,+13,+16[2]*/48,XY,+7,+13[7]*;	Severe
	Sigmoid colon	10	72,XXX[4]*/76,XXX[2]*/130,XXXXX[1]*;	Severe
	Rectum	11	84,XXXYYY[4]*/89,XXXXYYYY[1]*/95,XXXXYYYY[2]*;	Severe
	Rectum	12	47,XY,del (3p),+13[5]*;	Severe
	Sigmoid colon	13	47,XY,+13[3]*/48,+13,+18[2]*/49,XY,+13,+18,+20[2]*;	Severe
			FAP	
Tubular adenomas	Rectum	14	48,XX,+7,+13[7]*;	Severe
	Sigmoid colon	15	48,XX,+12,+16[2]*/52,XX,+3,+12,+16,+18,+19,+20[4]*/64,XXX[1]*/67,XXX[1]*;	Severe
	Rectum	16	47,XY,1p-,+7,+mar,-17/[3]*/65,XXXY,1p-,+7,+mar,-17/[9]*	Severe with areas of carcinoma *in situ*
	Rectum	17	47,XX,+7[5]*	Severe

Notes: 5–12 metaphase plates per specimen were analyzed. *Number of metaphase plates analyzed for each sample.

Table 2. Spectrum of chromosome abnormalities in single adenomas, in adenomas from patients with hereditary polyposis and in *in situ* adenocarcinomas according to own results and the literature data

Type of tumor	Aneuploidy		Structural chromosome rearrangements			
	Additional copies of chromosomesl	Additional copies of chromosomes II	Deletions I	Deletions II	Translocations I	Translocations II
Adenomas (sporadic form of polyps)	7, 8, 13, 14, 15, 16, 18, 20	1, 2, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, 19, 20, 22	3p, 8q	3p, 10q,	–	(3; 14); (10; 15).
Adenomas (hereditary form of polyps)	7, 12, 13, 16, 18, 19, 20	7, 8, 13	–	–	–	–
Adenomas with with areas of carcinoma *in situ* (sporadic form of polyps)	Modal quantity (78–130)	2, 7, 14	–	5p	–	–
Adenomas with infiltrating adenocarcinomas (hereditary form of polyps)	Modal quantity (47–65)	2, 3, 8, 9, 12, 13, 14, 19, 20	1p	–	–	–

Notes: I – own results; II – literature data [7–9].

We have observed different spectrum of chromosome abnormalities in the adenomas from patients with FAP. The chromosome abnormalities were found in 4 out of 9 (44.4%) patients with FAP. Three cases (from these 4) were represented by aneuploid sets with additional copies of the chromosomes 7, 12, 13, 16, 18, 19, 20 with different combinations. The aneuploid changes detected at early stages may underlie accelerated tumor progression, increased cancer risk and poor prognosis. FAP adenomas were characterized by gains affecting chromosomes 7 and 13 [12]. One patient with Gardner syndrome (the variant of FAP) had CRC complicated with hepatoblastoma; the karyotype of adenomas was mosaic with combination of different cell clones containing from 47 to 66 chromosomes, when the prevailing cell clone was near-triploid with modal chromosome quantity from 60 to 66 with fragments of deleted chromosomes of unknown origin and losses chromosome 1p, 17 (16, Table 1). The pedigree of family with FAP of patient with polyposis is shown on Fig. 2. The proband and his affected relatives carried the mutation c.3343delA in the *APC* gene. The diagnosis was confirmed using molecular genetic analysis in the Institute of Human Genetics of Polish Academy of Sciences.

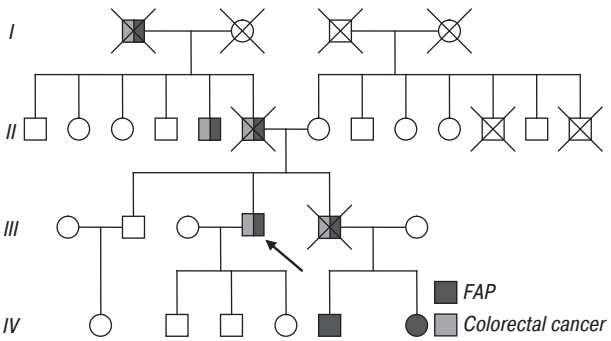

Fig. 2. The pedigree of family with FAP and colorectal cancer. The proband and his affected relatives (brother and 2 nephews) carried the mutation c.3343delA in the *APC* gene. The karyotype of the FAP adenoma (N16, Table 1) as follows: 47,XY,1p-,+7,+mar,-17/[3]/66,XXXY,1p-,+7,+mar,-17/[9]*

Comparison of the spectrum of chromosome abnormalities in single adenomas, adenomas from patients with hereditary polyposis and in adenocarcinomas *in situ* (according to our own results and the literature data) is shown in Table 2.

The chromosome abnormalities were found in 100% cases of adenocarcinoma bioptates. The localization of tumors in large bowel was as follows: rectal in 15 patients (83.3%); sigmoid, transversal and left colon localization had single patients (5.5% for each localization) (Table 3).

The quantity of the cell clones of tumors with alterated ploidy (near-diploid, near-triploid and near-tetraploid) were observed. The karyotype of adenocarcinomas had wide intratumoral chromosomal variations. During tumor progression many clones were generated. Anatomy and clinical data, and modal number of chromosomes in cell clones for each case are shown in Table 3.

Table 3. Clinical and chromosomal data from colorectal adenocarcinomas

Sex/Age	Localization of neoplasm	TNM classification of tumors	Modal number of chromosomes in cell clones
1.F/58	Rectum	pT1N0MoG1	51[2]/72[11]
2.M/59	Rectum	pT3N1M0G1	51-54[9]/96-104[2]
3. F/54	Rectum	pT3N1M0G1	43- 48[6]
4.M/76	Rectum	pT3N1M0G1	47[2]/58-64[21]
5.F/62	Transversum colon	pT2NoM0G1	67-78[13]/82-86[4]
6.M/72	Rectum	pT1N0M0G1	76-80[5]
7.F/54	Sigmoid colon	pT1N0M0G1	63-74[6]
8.M/54	Rectum	pT3N1M0G2	58-71[7]
9.F/51	Rectum	pT3N0M0G2	39-50[12]
10.M/69	Rectum	pT3N1M0G2	40-44[9]
11.M/58	Rectum	pT3N1M0G2	48-52[3]/65-72[5]
12.F/61	Rectum	pT3N1M0G2	40-42[2]/68-75[6]
13.F/61	Rectum	pT2N0M0G2	62-70[5]
14.M/75	Rectum	pT4N1M1G2	48-51[6]/68-72[4]/86-109[4]
15. F/45	Rectum	pT4N1M1G2	43-54[3]/62-70[3]
16.F/58	Rectum	pT4N1M1G2	50-57[6]/60-69[6]/96-112[3]
17.F/74	Left	pT3N1M1G3	41-43[10]
18.F/71	Rectum	pT3N1M1G3	41-45[16]

The analysis of metaphasal plates had shown that there were more near-triploid cell clones (< 3n, > 3n chromosomes, 92 clones), less near-diploid cell clones (< 2n, > 2n chromosomes, 83 clones), and the least were near-tetraploid cell clones and clones with higher ploidy (< 4n, > 4n chromosomes, 13 clones). Thus, we determined 5 polysomal, 5 monosomal and 8 mixed-type tumors. The types of ploidies in adenocarcinomas of large bowel are shown on Fig. 3.

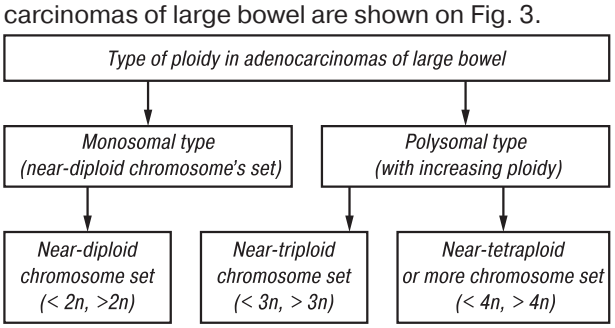

Fig. 3. The types of ploidy in adenocarcinomas of large bowel

Aneuploid chromosome sets were present in the karyotype of adenocarcinomas with additional copies of chromosomes 3, 16, 17, 18, 19, 20, 21, 22 in different combinations. Genomic instability is a crucial step in CRC progression and occurs in two ways. DNA mismatch repair deficiency leading to microsatelite instability (15% of cases). Genomic instability may occur at the chromosomal level and give rise to aneuploidy (85% of cases) [16].

Numerous chromosome rearrangements with high intratumoral heterogeneity of the karyotype, with three types of ploidy and numerous deleted chromosomes in the group of patients with poor prognosis have been determined. For example, patient 14 (see Table 3) had moderately-differentiated adenocarcinomas with low-differentiated areas and with disintegration set. We revealed a very heterogenous karyotype with 6 different cell clones in the specimens of adenocarcinoma, 4 of these are the following:

a) 48,XY,+16,+19,+20,-18[4]*/

b) 51,XY,+3,+13,+14,+16,+19,+mar1,+mar2,-17,-18[2]*/

c) 72,XXXYYY,+1(3),+2(6),+3(4),+5(6),+7(5),+10(4),+11(5),+12(3),+13(3),+14(3),+15(4),+16(3),+19(3),+20(4),+mar,-17,-18[2]*/

d) 109,XXXYYY,+1(4),+2(8),+3(4),+5(6),+6(3),del 6(?)q-, +7(7),+9(3),+10(4),+11(5),+12(4),+13(5),+14(5),+15(5), +16(4),+19(5),+20(10), +mar1(5),+mar2,-18,-8[2]*, where are numbers of analyzed clones.

There are many copies of some chromosomes (from 4 copies of chromosomes 1, 3, 6, 12, 16, Y to 5-7 copies of chromosomes 4, 5, 7, 11, 13, 14, 15, 19, to 8 — 10 copies of chromosomes 2, 20) with simultaneously numerous copies of pointed deleted chromosomes and with monosomy of chromosomes 8, 17, 18. According to the literature data, gain of 20q is observed in more than 65% of CRC. Gains of 20q are also common in other tumor types and have been associated with poor outcome in gastric cancer and CRC [15–18].

The microphoto of one clone with near-pentaploid karyotype is shown in Fig. 4.

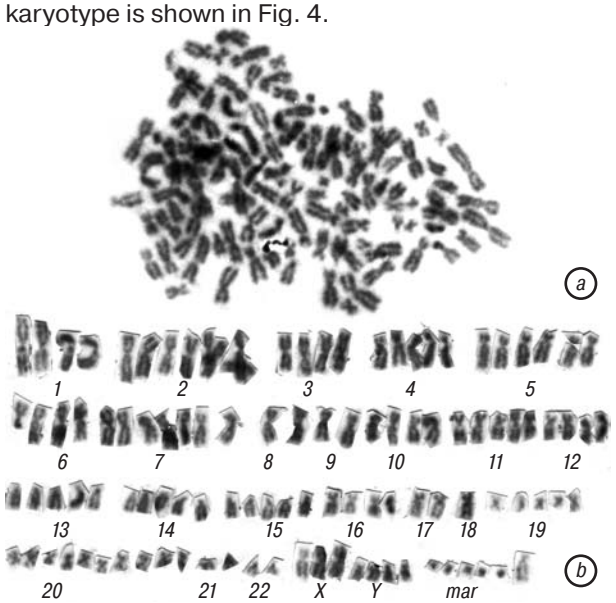

Fig. 4. Karyotype: 109, XXXYYY (cell clone N1). Moderately differentiated adenocarcinomas with low-differentiated areas and with seat of disintegration: *a*, metaphase plate; *b*, ideogram

The analogous heterogeneous karyotype with three types of ploidy and numerous deleted chromosomes were observed in another adenocarcinoma sample (case 16). Patient also had rectal polyp and adenocarcinoma of uterus and right ovary. The near-diploid cell clone with 56 chromosomes included trisomy of 12 chromosomes and other rearrangements: 56,XX, 1p-,+3,+16,+17,+18, +19,+19,+20,+20,+21,+21,+22,+mar,-12,-14.

The poor prognostic cytogenetic feature was an appearance of monosomal type of tumor with allelic losses of 18, 6, 11, 14 and X chromosomes (cases 17, 18) and the presence of numerous deleted chromosomes (case 15). Deletions of some chromosomes were found in 12 (66.7%) tumor samples. The size of deleted chromosomes was variable and fluctuated from small metacentric to acrocentric chromosomes, and sometimes their origin can't be established using G-method. The most frequently deleted chromosome of A-group was chromosome 1 (del 1p) (cases 16, 17).

Thus, the origin of tumors of large bowel is frequently characterized by alterations on the chromosomal level. The majority of the karyotypes of adenomas are normal, but in some tumors with severe dysplasia of epithelium the quantitative chromosome abnormalities and structural rearrangements were determined. The most typical chromosome abnormalities were numerical, such as trisomies of chromosomes 7, 13, 14, 16, 18, 20. Molecular genetic studies confirmed the arising of the genetic abnormalities during the progression from polyp to carcinoma. The gene involved in FAP was localized to 5q21 and was identified as APC. Molecular studies of adenomas have described allelic loss involving 5q, 18q, 17p. At the cytogenetic level chromosome losses in adenoma also occurs, but loss of 5q was detected only in few samples [12].

The combination of different malignant tumors (adenocarcinomas with adenomas) leads to the appearance of abnormal cell clones with additional copies of chromosomes 13, 18 and 20. The transition from tubular to tubular-villous and villous adenomas was accompanied by the increase of chromosome abnormalities quantity from 33.3% to 60,0% and widening of the spectrum of abnormalities (deletions, translocations). The quantity of the chromosome anomalies doesn't reach more than 40% cases for the hereditary form of polyposis, despite of domination of tubular adenoma with different degree of dysplasia. The features of malignant growth of adenomas are manifested by aneuploidy and chromosome deletions. The alteration of ploidy may also occur, although this phenomenon is comparatively rare in adenomas (polyploidy was present in 10% of cases).

Numerous adenocarcinomas possess polysomic karyotype with near-triploid or near-tetraploid or more chromosomal sets. Elevation of clonal variety, selective accumulation of the copies of some chromosomes with simultaneously monosomy of another and deletions are typical for adenocarcinomas. Allelic losses of chromosomes 18, 17, 6, 8, 11, 14 and X, appearance of additional copies of chromosomes 2, 3, 20, 16 and structural rearrangements (deletions) are of significant value in tumor progression. Monosomy and partial monosomy of chromosome 1, 3, 17, 18 led to tumor progression, due to the existence of recessive tumor suppressor genes, located on the 18q and 17p chromosomes. According to the literature data, the expression of oncogenes (*N-ras, ski*), located on the chromosome 1, also lead to cell malignization and CRC progression. The high probability of alterations in 27 genes on chromosome 3 confirmed to play the role in colorectal cancerogenesis [19].

In conclusion, the majority of chromosome abnormalities in colorectal adenomas were numerical, such as trisomies of chromosomes 7, 13, 14, 16, 18, 20. The adenomas with a high-grade epithelial dysplasia possess abnormal karyotype in all cases. The aneuploid changes, such as gains of chromosome 7, monosomy of 17 and 1p-, in FAP adenomas may lead to accelerated tumor progression, increased cancer risk and poor prognosis. The chromosome abnormalities were found in 100% of cases in adenocarcinoma bioptates. We determined 5 polysomal, 5 monosomal type and 8 mixed-type tumors. Numerous chromosome rearrangements with high intratumoral heterogeneity of the karyotype with three types of ploidy and numerous deleted chromosomes were determined in the group of patients

with poor prognosis. Appearance of monosomal type adenocarcinomas with allelic losses of chromosomes 18, 6, 11, 14 and X, and the presence of numerous deleted chromosomes were cytogenetic features of CRC with poor prognostic value.

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REFERENCES

1. **De la Chapell A.** Genetic predisposition to colorectal cancer. Cancer 2004; **4**: 769–80.

2. **Burt R, Neclason DW.** Genetic testing for inherited colon cancer. Gastroenterology 2005; **128**: 1696–716.

3. **Kaz AM, Brentnall TA**. Genetic testing for colon cancer. Natural clinical practice. Gastroenterol Hepatol 2006; **12**: 670–9.

4. **Svedsen LB, Thorup J, Larsen JK, *et al.*** Association between tumor DNA aneuploidy and *in vitro* tetraploidy of skin fibroblasts in patients with colorecral neoplasms. Scand J Gastroenterol 1989; **24**: 755–60.

5. **Lynch HT, Smyrk T, Lynch J, *et al.*** An update of HNPCC (Lynch syndrome). Cancer Genet Cytogenet 1997; **93**: 84–99.

6. **Nalieskina LA, Vorobjova LP, Choma DI, *et al.*** The features of P53 protein expression in the mucosal epithelium of large bowel, affected with polyps and cancer. Cytol Genet 2000; **34**: 15–22 (in Ukrainian).

7. **Ikeuchi T, Yoshida M, Iwana T, *et al.*** Chromosome abnormalities in colon carcinomas and adenomas from patients with familial polyposis coli. Cancer Genet Cytogenet 1989; **41**: 282, B28.

8. **Longy M, Saura R, Schouler L, *et al.*** Chromosomal analysis of colonic adenomatous polyps. Cancer Genet Cytogenet 1990; **49**: 249–57.

9. **Griffin CA, Lazar S, Hamilton SR, *et al.*** Cytogenetic analysis of intestinal polyps in polyposis syndromes: comparison with sporadic colorectal adenomas. Cancer Genet Cytogenet 1993; **67**: 14–20.

10. **Lozynska MR.** The spectrum of cytogenetic changes in the colorectal polyps with different histologic structure. Cytol Genet 2008; **6**: 398–403.

11. **Muleris M, Delattre O, Olchwang S, *et al.*** Cytogenetic and molecular approaches of polyploidization in colorectal adenocarcinomas. Cancer Genet Cytogen 1990; **44**: 107–18.

12. **Diep CB, Kleivi K, Ribeiro FR, *et al.*** The order of genetic events associated with colorectal cancer progression inferred from meta-analysis of copy number changes. Genes Chromosomes Cancers 2006; **45**: 31–41.

13. **Mitelman F, ed.** ISCN: an international system for human cytogenetic nomenclature. Basel: S Karger, 2005. 128 p.

14. **Lozynska MR.** The method for specimens preparation *in vivo* of metaphase chromosomes from adenocarcinomas of large bowel to determine the spectrum of cytogenetic changes during malignization of epithelium in tumors of large bowel: Patent no.34834; 2008 (in Ukrainian).

15. **Wilting SM, Snijders PJ, Meijer GA, *et al.*** Increased gene copy numbers at chromosome 20q are frequent in both squamous cell carcinoma and adenocarcinomas of the cervix. Pathol 2006; **209**: 220–30.

16. **Carvalho B, Postma CP, Mongera S, *et al.*** Multiply putative oncogenes at the chtomosome 20q amplicon contribute to colorectal adenoma to carcinoma progression. Gut 2009; **58**: 79–89.

17. **Postma CP, Hersen MA, Coffa J, *et al.*** Chromosomal instability in flat adenomas and carcinomas of the colon. Pathol 2005; **205**: 14–21.

18. **Aust DE, Muders M, Kohler A, *et al.*** Prognostic relevance of 20q 13 gains in sporadic colorectal cancers: a FISH analysis. Scand J Gastroenterol 2004; **39**: 766–72.

19. **Gerashchenko GV, Gordiyuk VV, Skrypkina I, *et al.*** Screening of epigenetic and genetic disturbences of human chromosome 3 genes in colorectal cancer. Ukr Biochim J 2009; **81**: 81–7 (in Ukrainian).