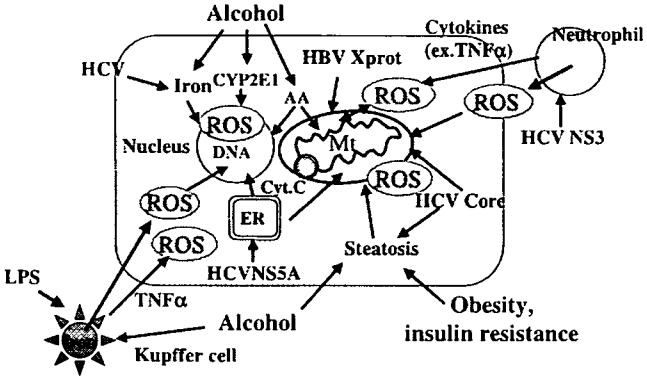

Fig. 3. A variety of etiologies enhance generation of ROS in hepatocytes. Overproduction of ROS through either endogenous or exogenous insults, is recognized to play an important role in the initiation and promotion events of hepatocarcinogenesis. See details in the text. *Mt*, mitochondria; *AA*, acetaldehyde; *LPS*, lipopolysaccharide; *ER*, endoplasmic reticulum; *Cyt. C*, cytochrome C

Conclusion

As described above, ROS in hepatocytes are generated endogenously through a variety of processes, and also can be derived from exogenous sources (Fig. 3).

A number of defense systems have evolved to combat accumulation of ROS. However, when these defense mechanisms are exhausted or overrun, the cellular redox potential shifts toward oxidative stress, in turn increasing the potential for damage to cellular nucleic acids, lipids, or protein. Although these events may be derived by different mechanisms, a commonality is the involvement of ROS in the development of HCC. In particular, unrepaired damage to DNA may result in mutations, provided that cell replication ensues prior to repair of modified bases. In addition to oxidative nuclear DNA damage, formation of mitochondrial DNA damage and mutation and alteration of mitochondrial genomic function have been revealed to contribute much to the process of carcinogenesis. At least three distinct stages of carcinogenesis, initiation, promotion and progression, have been identified. Aside from a role of oxidative stress in the induction of mutation, it is apparent that ROS and the cellular redox state mediate cell signaling pathways that are involved in cell growth and survival, leading to promotion and progression.

Therefore, oxidative stress is involved in all stages of carcinogenesis. As long as HCC develops mostly from chronic liver diseases, antioxidant therapeutic strategy and/or anti-inflammatory treatment might be required to prevent the development of hepatocarcinogenesis.

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Proportion of De Novo Cancers Among Colorectal Cancers in Japan

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Background & Aims: Adenomatous polyps are main precursors of colorectal cancers (CRCs). In Japan, de novo cancers, which do not arise from preexisting adenomas, are considered to account for a substantial number of CRCs, but the relative importance of de novo carcinogenesis remains controversial. This study estimated the proportion of de novo cancers among CRCs in Japan. *Methods:* The subjects were persons 40–79 years of age who were relatively similar to those in the general population. The subjects underwent colonoscopy between 1997 and 2001. Early cancers among CRCs detected in this study were classified as de novo cancers or polyp cancers derived from adenomas. The age-specific incidence of the early CRCs was calculated, and the proportion of de novo cancers was estimated. The lifetime risk of early CRCs was estimated. *Results:* The study group comprised 14,817 persons. CRCs were diagnosed in 189 subjects, including 83 early cancers. There were no differences with regard to size and location between de novo cancers and polyp cancers, but morphology differed. Eighty-four percent (16/19) of de novo cancers were flat elevated or depressed. The expected lifetime risk of developing early CRCs was 5.27% for men and 3.21% for women. Among persons with early cancers, the expected probabilities of developing de novo cancer were 18.6% for men, 27.4% for women. *Conclusions:* De novo cancers account for a considerable proportion in Japan. This information suggests that the recommended interval for colonoscopic examination in Japan should be shorter than that in the United States.

The incidence of colorectal cancer (CRC) has rapidly increased in Japan. At present, CRC is the fourth leading cause of death in males and the third in females.[1] Recent studies in the United States and Europe have shown that most CRCs develop from adenomatous polyps via the adenoma-carcinoma sequence; this theory is now widely accepted.[2,3] In 1977 in Japan, Kariya et al described a case of depressed cancer that did not arise from an adenomatous polyp.[4] Subsequently, Ishii et al,[5] Kudo et al,[6] and Shamsuddin et al[7] reported depressed cancers associated with invasion or metastasis, including some lesions less than 1 cm in diameter. These findings led to the theory that some CRCs develop by de novo carcinogenesis, rather than from adenomatous polyps. In Europe, the existence of small de novo cancers less than 1 cm in diameter with invasive properties was reported.[8] Several authors showed that de novo cancers lacked K-*ras* mutations on gene analysis.[9–11] Kaneko et al suggested that some small invasive cancers less than 2 cm in diameter and characterized by a nonpolypoid growth pattern and no K-*ras* mutations are due to de novo carcinogenesis.[12] These findings led to the hypothesis that de novo cancer may develop independently of the adenoma-carcinoma sequence.

CRCs developing from adenomatous polyps can be prevented by colonoscopic surveillance and treatment. Estimation of the impact of colonoscopic screening on public health requires an accurate estimate of the proportions of CRCs developing from adenomatous polyps and CRCs developing from de novo carcinogenesis. Several studies have attempted to quantify the proportion of de novo cancers among all CRCs, but estimates have ranged from as low as 3.8% to as high as 80%.[13–15] This wide discrepancy is attributed to differences in factors such as sample size, the definition of de novo cancer, and the criteria for the selection of subjects or study design. We estimated the proportion of de novo cancers among CRCs based on well-defined criteria in a large number of subjects with characteristics relatively similar to those of the general population.

Abbreviation used in this paper: CRC, colorectal cancers.

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Materials and Methods

Subjects

The subjects were persons who attended a gastroenterology clinic in Kumamoto, Japan, from 1997 through 2001. We excluded persons who had a history of polypectomy, mucosal resection, or surgery for advanced neoplasms (adenomas more than 10 mm in diameter, severe dysplasia, or cancer) within the previous 5 years; those who were referred for treatment by other clinics; and those with symptoms of bowel stenosis suspected to be caused by CRC. The study group finally comprised 14,817 persons 40 to 79 years of age who underwent total colonoscopy. When we found the area with a different color from surrounding mucosa, or slight deformity on the folds, we used chromoendoscopy or magnifying endoscopy technique during colonoscopy procedures to make the existence of a lesion or its appearance clear. The subjects had no gastrointestinal symptoms and underwent colonoscopy for screening or had a slight transient abdominal discomfort or a positive fecal occult blood test. All patients gave oral informed consent for this study, which was approved by the Ethics Committee of Hattori GI Endoscopy and Oncology Clinic.

Procedures

It is difficult to distinguish de novo cancers among advanced cancers when they are in the advanced stage of carcinogenesis because their shape has changed completely from early stage, all adenomatous components have been replaced by cancerous components, and they have accumulated genetic changes during their progression. Even Dukes A, stage I or early cancers cannot be classified as de novo or polyp cancer with morphologic appearance only because some de novo cancers become flat elevated or polypoid as they begin to invade. De novo cancers can be distinguished among early CRCs on the basis of growth pattern, existence of adenomatous components, and genetic changes.[12] De novo cancers among early cancers were diagnosed according to both of the following histologic criteria: (1) the absence of adenomatous components in the tumor and (2) all lateral margins of the tumor covered with normal mucosa and nonpolypoid growth pattern (Figure 1). All other cancers in this study were diagnosed as polyp cancers arising via the adenoma-carcinoma sequence because there remained some possibility of including unusual polyp cancers as de novo cancers if the only diagnostic criterion used were the absence of adenomatous components.

In accordance with Japanese guidelines, we defined early CRCs as cancer with mucosal or submucosal involvement and advanced CRCs as cancer with deeper involvement regardless of the presence or absence of lymph node metastases. Early CRCs were classified as either de novo cancer or polyp cancer, and the proportions of these lesions were calculated. The age distribution, size, location, and morphologic appearance of these lesions were also examined.

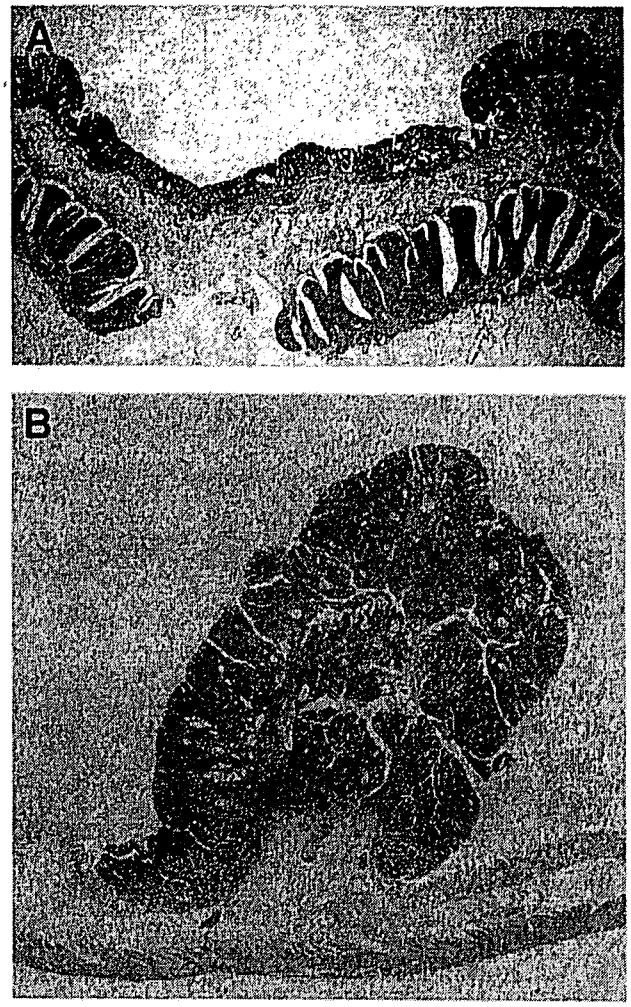

Figure 1. The histologic features of de novo and polyp cancer. (*A*) De novo cancer. The morphology is depressed type. Lateral margins are covered with normal mucosa. (*B*) Polyp cancer. Polyp includes adenomatous components.

Under the assumption that survival declines exponentially, we can convert a cumulative incidence to an incidence rate. This approximation, called the declining exponential approximation of life expectancy (DEALE) method,[16] was applied to estimate age and gender-specific incidence rates of early CRCs. The calculated incidence rates were compared with those of all stages of CRCs from the Surveillance, Epidemiology, and End Results (SEER) study in the United States.[17]

Method for Calculating Lifetime Risk

As only limited information is available for estimating the lifetime risk of early CRCs, we used a simplified approach to calculate the expected number of early CRCs and their subtypes (polyp cancer/de novo cancer) developing in persons 40 to 79 years of age.[18] The following formula was used to calculate the expected number of early CRCs in age category x.

Table 1. Chracteristics of Study Subjects

	Men (N = 6660)	Women (N = 8157)	Total (N = 14,817)
Age category, y			
40–49	1578	1636	3214
50–59	2152	2547	4699
60–69	1901	2410	4311
70–79	1029	1564	2593
Number of detected cancer	116	73	189
Early colorectal cancer	49	34	83
Advanced colorectal cancer			
≤20 mm	9	6	15
>20 mm	58	33	91
Total	67	39	106

$$a_x = (l_x - l_{x+1}) \times \frac{r_x}{m_x + r_x}$$

a_x: Expected number of early CRCs developing in age category x; l_x: number of survivors free of CRCs in age category x; r_x: incidence rate of early CRCs developing in age category x in persons free of CRCs in age category x-1; m_x: mortality rate excluding CRC-related deaths in category x.

To estimate the expected number of early CRCs developing from 40 to 79 years of age, we set l_x as 100,000 and calculated a_x for each age group (ages 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, and 75–79 years). The sum of the number for each age group was calculated to derive the total expected number of early CRCs. We also calculated the expected number of early CRCs from 40 to 79 years of age according to subtype (polyp cancer and de novo cancer). We used these data to estimate the age-specific rate of early CRCs and the proportion of de novo cancers. We also estimated the lifetime risk of all stage CRCs including early and advanced CRCs. Age-specific mortality rates excluding CRC-related deaths were derived from the Vital Statistics of Japan.[1]

Results

The characteristics of the subjects are shown in Table 1. The total number of subjects was 14,817 (men, 6660; women, 8157). There was no difference in age distribution between men and women. CRC was detected in 189 subjects (early cancer, 83; advanced cancer, 106); the incidence proportion was 1.74% (116/6660) in men, 0.89% (73/8157) in women, and 1.28% (189/14,817) overall. Of the 189 patients in whom CRCs were detected, 83 (44%) had early CRCs.

The characteristics of polyp cancers and de novo cancers detected among early CRCs are shown in Table 2. The proportion of de novo cancers was 22.9% (19/83). There were no differences with regard to size and location between them, but morphology differed. Eighty-four percent (16/19) of de novo cancers were flat elevated or depressed, whereas polyp cancers were literally polypoid.

The age-specific incidence proportions of early CRCs and their subtypes are shown in Table 3. The crude incidence proportion was 0.56% (men, 0.74%; women, 0.42%). The incidence proportions of polyp cancers and de novo cancers increased with age. In contrast to polyp cancers, de novo cancer was not found in the age 40–49-years group. The proportion of de novo cancers among all early CRCs was 22.9% (men, 18.4%; women, 29.4%).

The expected number of persons with early CRCs, ie, so-called lifetime risk, per 100,000 inhabitants 40 to 79 years of age in Japan and the estimated prevalence of de novo cancer are shown in Table 4. The expected lifetime risk of developing early CRC was 5.27% for men and 3.21% for women. Among persons with early CRC, the expected probabilities of developing de novo cancer were 18.6% (0.98/5.27) for men, 27.4% (0.88/3.21) for women, and 22.0% for the whole population.

The estimated incidence rates of early CRCs are compared with those of all stages of CRCs in the United States in Figure 2. Our data showed a higher incidence rate in the 40–55 year olds, followed by a gradual increase in the older age groups. This pattern differed from that in the United States, characterized by an exponential increase in the incidence rate of CRC with age.

Discussion

Estimation of de novo cancer is very important for setting the strategies for CRCs prevention and treatment and, simultaneously, is an issue very difficult to address correctly. To our knowledge, there have been few studies

Table 2. Characteristics of Early Colorectal Cancer

	Polyp cancer	De novo cancer	Percentage of de novo cancer
Age category, y			
40–49	10	0	0.0
50–59	18	4	18.2
60–69	20	9	31.0
70–79	16	6	27.3
Total	64	19	22.9
Size, mm			
≥20	17	5	
>10	26	7	
≤10	21	7	
Location			
Right side	22	6	
Left side	27	6	
Rectum	15	7	
Morphology			
Polypoid	64	3	
Flat elevated	0	6	
Depressed	0	10	

Table 3. Age-Specific Incidence Proportion of Early Cancer and Their Subtype

Age category, y	Number of subjects	Type of cancer		Proportion of De novo cancer in the total cases (%)	Incidence proportions of cancer (%)			
		Polyp cancer	De novo cancer		Polyp cancer	De novo cancer	All	SE
Men								
40–49	1578	5	0	0.0	0.32	0.00	0.32	0.001
50–59	2152	14	1	6.7	0.65	0.05	0.70	0.002
60–69	1901	12	5	29.4	0.63	0.26	0.89	0.002
70–79	1029	9	3	25.0	0.87	0.29	1.17	0.003
Total	6660	40	9	18.4	0.60	0.14	0.74	0.001
Women								
40–49	1636	5	0	0.0	0.31	0.00	0.31	0.001
50–59	2547	4	3	42.9	0.16	0.12	0.28	0.001
60–69	2410	8	4	33.3	0.33	0.17	0.50	0.001
70–79	1564	7	3	30.0	0.45	0.19	0.64	0.002
Total	8157	24	10	29.4	0.29	0.12	0.42	0.001
Total								
40–49	3214	10	0	0.0	0.31	0.00	0.31	0.001
50–59	4699	18	4	18.2	0.38	0.09	0.46	0.001
60–69	4311	20	9	31.0	0.46	0.21	0.67	0.001
70–79	2593	16	6	27.3	0.62	0.23	0.85	0.002
Total	14,817	64	19	22.9	0.43	0.13	0.56	0.001

SE, Standard error.

that estimated the incidence proportion of early CRC in the general population based on the results of colonoscopy in a representative number of subjects. Because the colonoscopic examination fee in Japan is considered reasonable (approximately 136 US dollars), this is often included in medical checkups and screening of the general population.

Given that repeated colonoscopic examinations at appropriate intervals allow us to detect most CRCs at a very

Table 4. Expected Number and Probability of Persons With Early Colorectal Cancer From 40 to 79 Years of Age in Japan

	Expected number of persons developing early colorectal cancer in 100,000 people			
	Total		De novo type	
	Men	Women	Men	Women
Age category, y				
40–44	316.9	305.6	0.0	0.0
45–49	313.7	303.8	0.0	0.0
50–54	679.4	270.9	45.3	116.1
55–59	661.8	267.7	44.1	114.7
60–64	816.4	477.2	240.1	159.1
65–69	771.7	466.2	227.0	155.4
70–74	919.0	576.7	229.7	173.0
75–79	790.6	541.8	197.6	162.6
Total	5269.4	3210.0	983.9	880.9
Expected probability of developing early colorectal cancer (%)	5.27	3.21	0.98	0.88

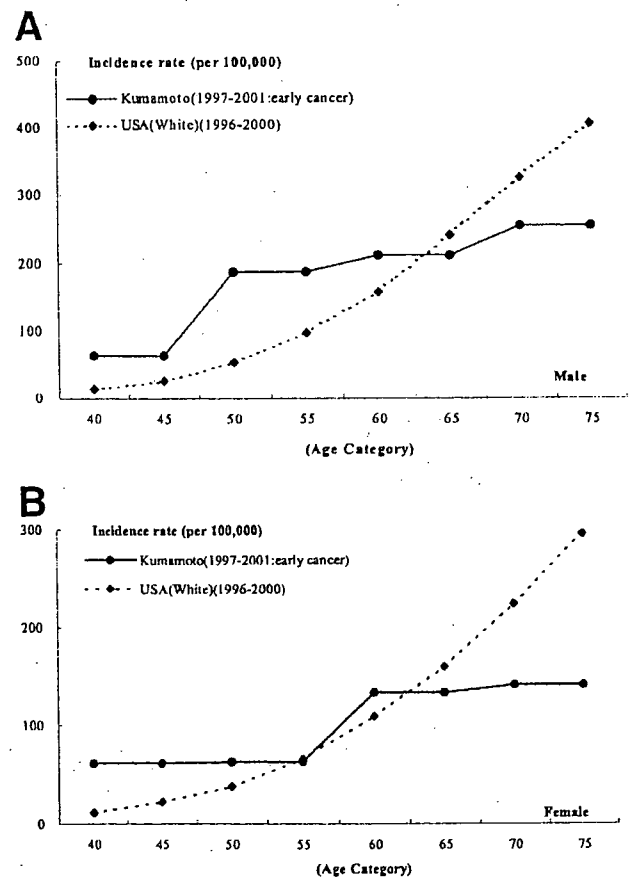

Figure 2. Age-specific incidence rates of early CRCs in Kumamoto were estimated by the DEALE method, and those rates of all stages of CRCs in the United States (*white*) were obtained from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute.

early stage, the data can be used to predict the lifetime risk of CRC. In this study, we excluded the detection rate of advanced CRCs from the estimated lifetime risk of CRC because such cancers would probably have been detected at an earlier stage if colonoscopy had been done at appropriate intervals. In our model, such advanced cancers are included in the incidence of early CRC several years before their detection as advanced CRCs. The lifetime risk of CRC was 11.4% for men and 6.29% for women when advanced CRCs were taken into account. That was much higher than the SEER data of 6.31% for men and 5.94% for women and supports the usefulness of our model.

At present, colonoscopy is the best means to detect early CRCs. However, de novo early cancers are considered difficult to detect even by colonoscopy because the protrusion is inconspicuous.[19,20] One study has described an early cancer initially detected while it still was 6 mm in diameter that was not reconfirmed on several subsequent colonoscopic examinations, only to be detected after progression to an advanced cancer (18 mm in diameter) 3 years 4 months later.[21] On the other hand, colonoscopy has enabled the detection of some small advanced cancers 2 cm or less in diameter that were difficult to detect by barium enema because they were flat or flat elevated or depressed.

In this study, the majority of de novo cancers were flat elevated or depressed, whereas all polyp cancers were literally polypoid. At the same time, small advanced CRCs 2 cm or less in diameter accounted for 14% (15/106) of advanced CRCs. According to our criteria of de novo cancer, 80% (12/15) of small advanced CRCs were de novo. Thus, the proportion of de novo cancers might be underestimated in our study because it is difficult to detect most of them in the stage of early CRCs, even if colonoscopy is carried out at appropriate intervals.

The characteristics of our subjects were assumed to be relatively similar to those of the general population. The important risk factors for CRC such as family history were not considered because such risk factors among the general population are unknown in Japan. Instead of risk factors, the reason for a colonoscopic examination was considered. The most common reason was screening with no symptoms, slight transient abdominal discomfort, or anxiety about cancer. The medical conditions in Japan are such that people can easily undergo colonoscopic examinations at a reasonable cost. In addition, our target in this study was early asymptomatic CRC. Persons with positive fecal occult blood tests, who accounted for approximately 10% of our subjects, were included because the fecal occult blood test is a screening technique with a high sensitivity but a low specificity for CRCs, especially advanced CRCs. The inclusion of persons with positive fecal occult blood tests was therefore considered not to influence the detection of early cancers.[22] Patients who had received treatment for advanced neoplasms during the past 5 years were excluded from the study because such patients are at a decreased short-term risk and at an increased long-term risk for developing CRC.[23] The extrapolation of our data to the general population has some limitations.

The natural history of tumors cannot be followed in humans for ethical reasons. There is no way to know whether small early cancer stays small, resolves spontaneously, or goes on to become symptomatic. The origin of tumors can only be inferred on the basis of indirect methods. Most early CRCs are thought to retain their initial structural features. The origin of such early cancers can therefore be inferred on the basis of histologic appearance. Our criteria of de novo CRC might not be agreed with in Western countries. However, we believe our criteria to be most credible because the changes in the morphology, the replacement of adenomatous components by cancer components, and the genetic changes occur during the progression from an early to an advanced cancer.

Kaneko et al have demonstrated that carcinomas showing the nonpolypoid growth characteristic of small invasive CRCs do not contain K-*ras* mutations, a characteristic of adenomatous polyps. Such carcinomas therefore probably do not derive from adenomatous polyps and are most likely de novo cancers.[12] In accordance with this hypothesis, we considered carcinomas showing nonpolypoid growth pattern with none of the features of carcinomas derived from polyps to be de novo cancers. We believe that it is the most practical method to estimate the proportion of de novo cancers among patients with early CRC.

To estimate the lifetime risk of CRC and proportion of de novo cancer, we used a simplified SEER method to apply our age-specific data. CRC incidence rate, CRC mortality rate, and total mortality rate except CRC mortality rate were collected from the literature. To account for the competing risk, CRC mortality rate and total mortality rate except CRC mortality rate were treated separately in the calculation of lifetime risk of CRC. The lifetime risk of CRC was calculated by summing up age-specific incidence of CRC until the age of 80 years. Although the resulting method was not exactly the same as the SEER technique, there was no major problem in practical use.

The SEER data showed that the lifetime risk of CRC per 100,000 residents in the United States was 6.31% for

men and 5.94% for women.[17] In Japan, the incidence of CRC has increased more than 4 times during the past 20 years.[1] Our estimates of the lifetime risk for developing early CRC from 40 to 79 years of age (5.27% for men and 3.21% for women) were similar to the SEER estimates, especially in men. Similar risk levels might reflect the increasing Westernization of lifestyle in Japan or might support that some of the early cancers do not grow and do not become symptomatic or fatal. Our data showed higher incidence rates in middle age and lower incidence rates in advanced age as compared with the SEER data. The SEER data may reflect mainly the incidence of advanced CRCs. The differences in age-related incidence rates between our results and the SEER data, despite similar lifetime risks, might be associated with the time interval required for progression from early to advanced CRCs. Verification of this assumption would provide evidence that repeated colonoscopy at appropriate intervals is useful for the detection of most CRCs at an earlier stage, which can be cured by appropriate intervention.

Our results showed that de novo cancers account for approximately 22% of early CRCs. The estimation of our results is limited for the reasons described above. Estimation of de novo cancer is very important for setting the strategies to prevent and treat CRCs, and, simultaneously, it is a very difficult issue.

Our risk estimate was lower compared with that obtained in a simulation study carried out in Taiwan (30%).[24] When analyzing early CRCs and small advanced CRCs ≤2 cm, 30% of these cancers were de novo. This high prevalence of de novo cancer among small advanced CRCs might be attributed to the difficulty in identifying de novo cancer as early CRC because of morphologic reasons or rapid growth. In this study, we used chromoendoscopy or magnifying endoscopy technique when the existence of a lesion is suspected. Repeated colonoscopy at appropriate intervals or another new technique, such as fluoroscopy or narrow-band images, might be needed to detect most de novo cancers in the stage of early CRCs.

The National Polyp Study[25] conducted in the United States proposed that follow-up examinations after colonoscopic removal of newly diagnosed adenomas should be done every 3 years. If only 1 or 2 small (<1 cm) tubular adenomas are detected, follow-up examinations should be done every 5 years.[23] These proposals are evidence based as well as practical. However, the results of our study suggest that de novo cancers account for 22% of early CRCs, even after resection of all adenomatous polyps. Available evidence suggests that the recommended interval for colonoscopic examination in Japan should be shorter than that in the United States. The Japan Polyp Study, a large, multicenter, randomized, controlled study organized by investigators at the National Cancer Center that includes our hospital has been initiated to determine the characteristics of CRC and the most cost-effective surveillance intervals for colonoscopy in Japan.[26] That will show another aspect of the magnitude of the risk and incidence proportion of de novo cancer in Japan. Information on de novo cancer will contribute to the determination of the appropriate interval for colonoscopy and to establish the strategies for the prevention and treatment of CRCs.

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第2章 肝癌発生の実態と発癌機序

4．肝細胞癌における遺伝子異常

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〈Key point〉

はじめに

肝発癌増殖進展過程では，慢性肝炎，肝硬変を経て前癌病変である腺腫様過形成あるいは過形成結節が生じ，早期肝細胞癌(hepatocellular carcinoma；以下，HCC)から進行HCCへと移行する．本邦においてHCCの約90%はウイルスの持続感染を基礎疾患として有しており，持続感染による炎症が遺伝子異常をはじめとしたさまざまなメカニズムを介して発癌進展に関与すると考えられている．また，B型肝炎ウイルス(以下，HBV)やC型肝炎ウイルス(以下，HCV)が宿主遺伝子の機能変化をもたらし，発癌に関与する可能性もある．

本稿ではこれまでに明らかになっているHCCにおける遺伝子異常を，Weinbergらにより提唱された多段階発癌の概念[1]と対比しながら，肝炎ウイルスの関与を交えつつ概観する．

Key words：肝細胞癌，遺伝子異常，LOH，メチル化，肝炎ウイルス

Genetic Alterations in Hepatocarcinogenesis

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Ⅰ．肝発癌の各段階に認められる遺伝子異常

1．増殖シグナルの自己増強(癌原遺伝子などの変異による)

癌原遺伝子や細胞周期関連遺伝子に変異が生じると増殖シグナルの自己増強が起こるが，一般に認められる変異は点突然変異と遺伝子増幅とに大別される．

点突然変異

点突然変異では，塩基の一つが正常とは異なる塩基に置き換わる(図a)．例えば，癌原遺伝子のなかで *ras* 遺伝子ファミリーである *H-*，*N-*，*Ki-ras* に点突然変異が起こると，増殖シグナルは恒常的に活性化される．しかしながら，*ras* 遺伝子ファミリーの活性化変異はHCCでは数%とその頻度は低い．

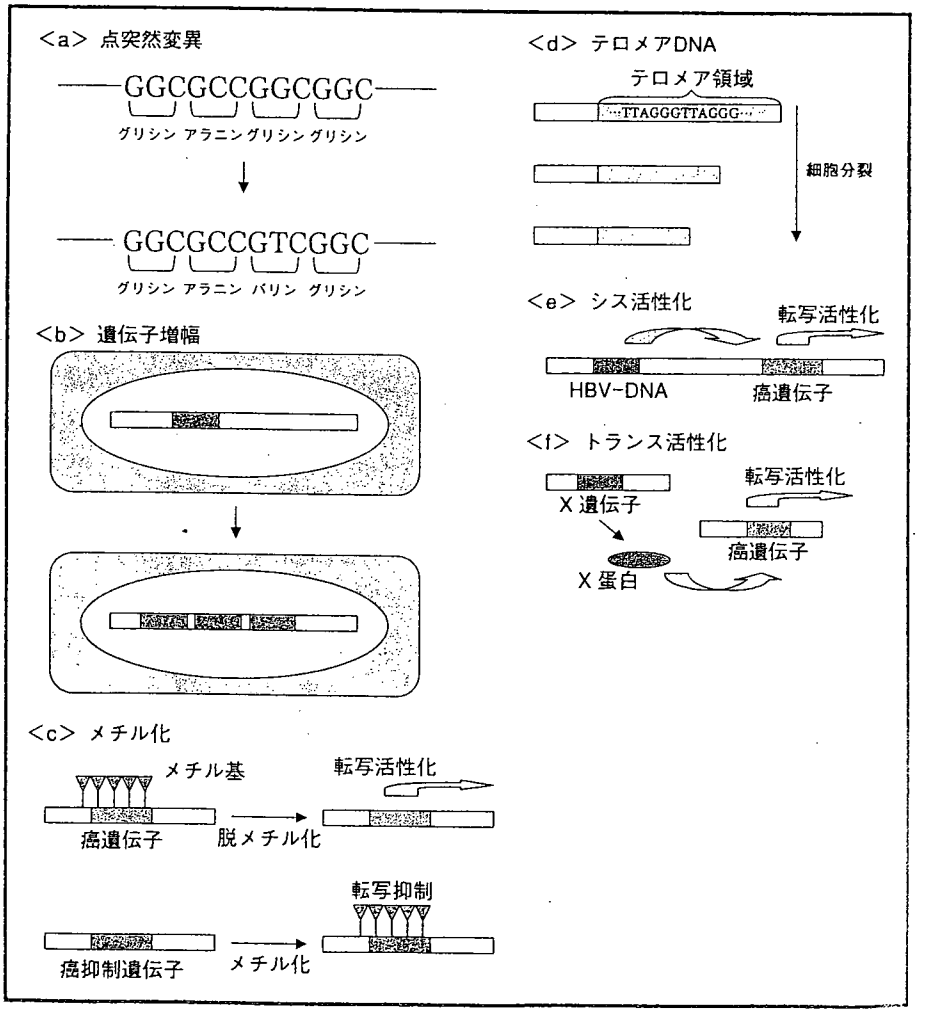

図　肝細胞癌の遺伝子異常

一方，遺伝子自体の構造には異常がなく，特定の遺伝子を含む染色体領域が多数コピー増幅され，そのために遺伝子発現が大幅に増強している場合を「遺伝子増幅」と呼ぶ(図b)．癌原遺伝子 *c-myc* 遺伝子の存在する8q24はHCCの40%で2～5倍の獲得が観察され，*c-myc* 遺伝子自体にも遺伝子増幅が報告されている[2]．

遺伝子増幅

一方，G_1期からS期への進行を制御する細胞周期関連遺伝子サイクリンD_1は，2～10倍の遺伝子増幅と6～10倍の過剰蛋白発現が進行HCCにおいて約10%の頻度で報告されている[3]．またインスリン，インスリン様増殖因子1(IGF-1)の増殖シグナルに関与するインスリン受容体基質(IRS-1)[4]でも遺伝子増幅が認められる．一方，β-カテニンはWntシグナル伝達を担い，*c-myc* 遺伝子やサイクリンD_1遺伝子などの転写を誘導する癌原遺伝子であるが，早期HCCにおいてβ-カテニン遺伝子の変異が報告されている[5]．

2．増殖抑制シグナルに対する不応性(癌抑制遺伝子などの変異による)

癌抑制遺伝子は正常細胞で発現しており，増殖シグナルを抑える働きをしている．通常，正常組織の遺伝子座において，父方由来と母方由来の対立遺伝子は同じでないが，この一方が欠失してホモ接合体に変化している場合をヘテロ接合性の消失(loss of heterozygosity；以下，LOH)と呼ぶ．LOHの頻度は染色体部位にかかわらず，概して進行癌において頻度が高くなり，前癌状態である腺腫様過形成あるいは過形成結節の段階では散在性に認めるにとどまり，特定領域にLOH集積の報告はない．

LOH

一方，慢性肝炎から直接にHCCが発生する症例では，LOHの集積を1p，6q，8p，13qの部位に認める頻度が高い[6]．また早期HCCでは1p，4q，6q，8pなどでのLOHの頻度が高い．

1pのLOHは2cm以下の早期HCCでテロメア側に比較的高頻度に認められる．この部位には癌抑制遺伝子 *p53* と相同性を有する *p73* 遺伝子が存在するが，HCCにおいて *p73* 遺伝子変異はまれであり，p73蛋白質の発現はむしろ増強していることから[7]，p73の不活化はHCCと関連が乏しい可能性がある．

6q25-27に存在するマンノース6-リン酸/インスリン様増殖因子-II受容体(以下，M6P/IGF2R)は，TGF-βを不活性型から活性型へ変換する働きを有しており，増殖抑制に働く．M6P/IGF2Rの変異の大部分は肝細胞の異形成領域で検出され[8]，HCCの早期に機能欠損を介した増殖亢進への関与が示唆される．

M6P/IGF2R

進行HCCでは，1p，4q，6q，8pに加え，13q，16q，17pなどにLOHの報告が多い．HCCの進展過程でLOHを認める染色体部位が増加することから，複数の遺伝子(とくに癌抑制遺伝子)に多段階に変異が蓄積すると考えられている．13qには癌抑制遺伝子*Rb*が存在しており，進行HCCにおいてLOHのみられる残存アレルに高率に点突然変異を認め，その機能欠損が明らかになっている[9]．

癌抑制遺伝子 *p53*

癌抑制遺伝子*p53*のLOHが17pに報告されている[10]．p53蛋白質はDNA傷害時に細胞周期を停止させDNAを修復するが，修復が不可能な場合にはアポトーシスを誘導する．*p53*遺伝子の変異は前癌状態や早期癌では報告がなく，むしろ分化度の低下，腫瘍径の増大に伴い変異の頻度が高くなることから[11]，癌の発生ではなく進展に関与すると考えられている．その他，癌抑制遺伝子の変異としては*PTEN*，*CDKN2A*(*$p16^{INK4A}$*)などがある[12]．

異常メチル化

一方，癌抑制遺伝子は，プロモーター領域の異常メチル化によっても機能が抑制される．哺乳動物におけるDNAのシトシン(C)の一部はメチル化されており，とくにCpG部位(CとGが連続した場所)の約80%はメチル化されている．一般的にメチル化はその領域に存在する転写単位の発現を抑制する．メチル化に異常が生じると細胞の癌化が引き起こされるが，その機序として，①メチル化により発現が抑制されていた癌遺伝子が，その低下により過剰に発現する，②通常発現している癌抑制遺伝子が異常メチル化により不活性化される，などが考えられる(図c)．例えば，癌抑制遺伝子*$p16^{INK4A}$*はサイクリン依存性キナーゼ4とサイクリンD_1との複合体形成を阻害し，細胞周期をG_1期に停止させる働きがあるが，早期HCCにおいてプロモーター領域の高頻度のメチル化によりp16蛋白質の発現が低下している[13]．

3．アポトーシスの回避

アポトーシス回避能

通常，遺伝子異常をきたした肝細胞はアポトーシスにより排除されるが，なんらかの機序でアポトーシスからの回避能を獲得すると，クローナルに増殖して癌組織へと進展する．

この回避能の分子機構として，アポトーシス実行機構の障害や制御機構の活性化が挙げられる．

アポトーシスの実行にはFasリガンド(以下，FasL)やTNF-αなどのdeath factorが中心となる．例えば，活性化T細胞に発現するFasLが細胞表面上のFasに結合すると，カスパーゼと呼ば

れる蛋白質分解酵素が活性化されアポトーシスが惹起される．一方，制御因子であるBcl-2ファミリーのなかで，アポトーシス抑制因子(Bcl-2，Bcl-XL)の増強と促進因子(Bax，Bad，Bidなど)の減弱が相まってアポトーシス回避能がもたらされるが[14]，HCCにおけるアポトーシスからの回避能と遺伝子変異との関連は十分には解明されていない．p53蛋白質はFasやBaxの転写を介してアポトーシスを誘導する．前述のようにHCCでは*p53*遺伝子が存在する17pに高頻度にLOHが認められ，さらに残存アレルでFasやBaxの誘導に必要なドメインⅣ，ドメインⅤに点突然変異が集まるために[11]，p53蛋白質によるアポトーシスの誘導は著しく阻害されている．

4．テロメラーゼの活性化

テロメア

真核生物の染色体末端(テロメア)にはテロメアDNAが位置しており(図d)，その長さは細胞分裂に伴い短くなる．一方，生殖細胞，癌細胞ではテロメアDNAを延長する酵素であるテロメラーゼの働きにより，テロメアDNAの長さが保たれている．HCCにおいても83%の頻度でテロメラーゼ活性の陽性が確認されているが，中低分化HCCほど陽性率は高い傾向にある[15]．

5．腫瘍血管新生

血管上皮増殖因子(vascular endothelial growth factor；以下，VEGF)や塩基性線維芽細胞成長因子(basic fibroblast growth factor；以下，bFGF)などは，腫瘍血管の新生に非常に重要な働きをする．実際にHCC患者血清やHCC組織，あるいはその周辺の非癌部組織でVEGFの濃度が高いこと[16]，術前の血清中のbFGF濃度が高値を示す患者は切除後の予後が不良であること，進行HCC患者ではbFGF濃度は高値であることが報告されている[17]．

VEGF

bFGF

また悪性腫瘍では正常組織に比べVEGFやbFGFの発現が増加し，逆に内因性阻害因子であるトロンボスポンディン1(以下，TSP-1)やβ-インターフェロンの発現が低下している．TSP-1はp53蛋白質によってその発現が制御されており[18]，*p53*遺伝子の変異によりTSP-1濃度が低下し，その結果，血管新生因子の作用が相対的に強くなる可能性が考えられる．

6．組織浸潤と転移

第16染色体のLOH

第16染色体のLOHは，HCCの腫瘍径，分化度，転移との間に関連が認められること，また進行HCCに高頻度に観察されることから，この染色体上に進展転移に関与する遺伝子の存在が示唆されている[19]．例えば，16qに存在する細胞接着因子E-カドヘリン遺伝子は異常メチル化の標的遺伝子であり，16qのLOHに加えてプロモーター領域のメチル化異常を介したカドヘリン機能低下が浸潤転移の一翼を担っている[20]．

E-カドヘリン

II．肝炎ウイルスと遺伝子異常

1．HBVによる宿主遺伝子の変化

HBV感染によってもたらされる宿主遺伝子変化のメカニズムとしては，①insertional mutagenesis，②トランス活性化機能，③*in vitro* recombination(genomic instability)が挙げられる．

1）insertional mutagenesis

HBVはウイルス自体がコードする逆転写酵素の働きで宿主染色体へ部分欠失した形で組み込まれるが(insertional mutagenesis)，慢性肝炎組織ではほぼ全例にHBV DNAのクローナルな組み込みが観察される[21]．HBV DNAが宿主ゲノムの癌原遺伝子や細胞周期関連遺伝子の近傍に組み込まれた場合に，それらが活性化され発癌がもたらされると考えられており，このような遺伝子の活性化機構はシス活性化(図e)と呼ばれている．

シス活性化

これまでは，染色体上のHBV DNAの組み込み部位はrandomであると考えられてきたが，近年，多数の症例の検討から，insertional mutagenesisによるシス活性化はまれではないことが示唆されている[22]．とくにテロメア合成に関与するヒトテロメラーゼ逆転写酵素(*hTERT*)遺伝子のpromoter領域への組み込みが報告されており[23]，組み込み部位がrandomでない可能性が示されている．

2）トランス活性化機能

トランス活性化

トランス活性化とは，その遺伝子が存在するDNA以外の遺伝子を活性化させることである(図f)．例えば，HBVのX蛋白は，細胞増殖に関与するRNAポリメラーゼII，IIIや*c-myc*，*c-fos*の遺伝子の転写をトランスに活性化する[24]．また，p53蛋白質の核内への移行を阻害して不活性化することや，またカスパーゼ3の活性を抑えてアポトーシス回避に働くこと[25]，などが明らかである．ま

た増殖シグナルの恒常的活性化がHCCで報告されているが[26]，X蛋白はRas/MAPキナーゼカスケードを活性化する[27]．一方，*X*遺伝子のトランスジェニックマウスでは高率にHCCが発症する[28]．このようにX蛋白の発癌誘導作用が示唆されているが，ヒトHCCにおけるX蛋白の発現は非癌部，癌部ともに50%前後であり[29]，発癌進展にどの程度関与しているかについては未だ明らかではない．

HBV X蛋白

3） ***in vitro* recombination (genomic instability)**

HBV DNAのpre C領域の61塩基断片(15 AB配列)が宿主DNAの再編成(recombination)を誘導し[30]，染色体の不安定性(genomic instability)をもたらす可能性が考えられる．

2．HCVによる宿主遺伝子の変化

HCVは逆転写酵素を有さないために，宿主遺伝子への組み込みは認められず，そのためにHBVのようなinsertional mutagenesisや*in vitro* recombinationを介した発癌誘導は当てはまらない．むしろコア蛋白は，①トランスに働き*c-myc*プロモーターを活性化する，細胞周期を停止させる$p21^{Waf1/CIP1/SDI1}$のプロモーター活性を抑制する[31]，②転写因子であるNF-$\kappa\beta$やAP-1の機能を変化させ，細胞増殖や細胞免疫を修飾する[32]，などの報告があり，HCVはHBVとは共通した，あるいは特異的な機構で発癌進展に関与しており，今後の研究が期待される．

HCV コア蛋白

3．細胞内環境の変化

HCVコア遺伝子トランスジェニックマウスの肝臓，あるいはC型慢性肝炎例ではC18：1不飽和脂肪酸が増加しており，そのため，脂肪酸代謝が過度に亢進し多量の活性酸素が産生される[33]．その結果，遺伝子変異が起こりやすくなると考えられている．実際に酸化的DNA損傷産物である8-hydroxy-deoxyguanosine(8-OHdG)は，慢性ウイルス性肝疾患においてその発現が増強している[34]．

活性酸素

8-OHdG

おわりに

HCCも他の癌と同様に多段階の発癌機構が想定されているが，HCCに特異的な遺伝子異常は未だ見出されていない．HCC発症の背景として，肝炎ウイルスの持続感染による慢性炎症が関与して

いることは疫学的にも明らかであるが，この慢性炎症から早期HCCに至る機構を現在の遺伝子異常の知見のみで説明することは不可能である．HCCの早期発見，高い再発率の抑制，分子標的治療の開発など，HCC撲滅にむけた臨床応用につなげるためにも，遺伝子異常の詳細な解明が待望される．

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