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Guest editorial *

Molecular and cellular features of esophageal cancer cells

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Abstract. More than 70 cell lines were established from esophageal cancer, including 15 TE-series cell lines established by the authors. This article reviews molecular and cellular features of esophageal cancer cells from studies using these cell lines as well as primary tumors. The subjects reviewed include primary cultures of normal epithelium of the esophagus and of esophageal tumors, their growth and differentiation properties, chromosomal aberrations, protein kinase C, growth factors and their receptors, oncogenes, and tumor-suppressor genes. Lesions of genetic loci in esophageal cancer include the absence of mutations in *ras* genes in primary tumors, amplification and overexpression of the c-*erb*B gene, co-amplification of *hst*-1 and *int*-2 genes, mutations, and allelic loss of tumor suppressor genes, p53, Rb, APC, and MCC. Future clinical improvement will be achieved on the basis of the understanding of molecular and cellular features of esophageal cancer cells.

Key words: Esophageal cancer – Cell lines – Oncogenes – Tumorsuppressor genes – Growth factors

Introduction

Esophageal cancer is the sixth most common cancer of the male population in Japan. In 1989, about 5600 people died of this cancer, constituting 4.6% of the total number of cancer deaths in Japan. Although the number of people who die from esophageal cancer is increasing, age-adjusted mortality rates have been relatively constant since 1950 in the range 6.67–7.76/100 000.

The mortality rate of esophageal cancer varies extremely throughout the world (Tomatis et al. 1990). The high-risk areas of the world include the so-called Asian esophageal cancer belt from the Caspian littoral in northern Iran, through the southern republics of the former Soviet Union (Turkmenistan, Kazakhstan and Uzbekistan) to western and northern China; southeastern Africa, parts of eastern South America (southern Brazil, Uruguay, Paraguay, northern Argentina); and certain defined areas of western Europe (France and Switzerland). In Linxian, China, the mortality rate is estimated to be as high as 211.2/100 000 in the male and 136.5 in the female population (Lu et al. 1985). There are also quite marked differences between ethnic groups in the USA and Singapore (Tomatis et al. 1990). These geographical differences suggest the importance of certain local dietary and cultural practices in the etiology of this cancer in high-risk areas. Dietary *N*-nitrosamines are probably a major risk factor in China (Singer et al. 1986; Bartsch et al. 1983). In other parts of the world, including Japan, tobacco and alcohol, particularly in combination, are prevalent risk factors (Tomatis et al. 1990).

The prognosis of esophageal cancer is poor, especially if metastasis takes place. Because of the lack of serous membrane in the outer surface of the esophagus, esophageal cancer rapidly invades surrounding tissues. Furthermore, it often metastasizes to regional lymph nodes and distant organs such as the liver, lungs, bones and brain. Patients die within 12 months unless radical surgery is performed. Even with surgical treatment, the prognosis is poor, the 5-year survival rate being only 25% after surgery in major hospitals in Japan.

An understanding of cellular and molecular features of esophageal cancer cells is essential for the improvement of clinical achievement. Thus, we have been investigating biological features of esophageal cancer cells using cell lines derived from esophageal cancer. Since the first report in 1976, 15 cell lines have been established from this cancer in the Second Department of Surgery, Tohoku University School of Medicine, Sendai. They are now known as the TE-series cell

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Abbreviations: PKC, protein kinase C; PCR, polymerase chain reaction; O^6-MedG, O^6-methyldeoxyguanosine
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lines and have been used worldwide as a model for investigating biological behavior in vitro, thereby contributing to our understanding of esophageal carcinogenesis. In the present article, we review molecular and cellular features of esophageal cancer cells obtained by the use of the TE-series and other cell lines, as well as primary tumors.

Culture of normal epithelium of the esophagus

For investigating molecular and cellular features of malignant counterparts, culture of normal epithelium of the esophagus is essential. However, the growth of epithelial cells is often interrupted by the overgrowth of fibroblasts. This problem was initially overcome by the introduction of a feeder layer of lethally irradiated 3T3 cells, which prevents fibroblastic overgrowth and promotes growth of keratinocytes (Rheinwald and Green 1975). Using the 3T3 feeder layer, it became possible to grow normal human esophageal epithelial cells (Banks-Schlegel 1985; Banks-Schlegel et al. 1985; Grace et al. 1985; Burg-Kurland et al. 1986).

We have established a method for isolation and cultivation of normal human esophageal epithelium in serum-free hormone-supplemented medium, i.e., modified RITC80-7 medium (Katayama et al. 1984, 1986). Sasajima et al. (1987) also cultured human esophageal cells in serum-free LHC-8 medium. As shown in Fig. 1, cultured esophageal cells show the typical morphology of epithelial cells forming a monolayer sheet.

The requirement for growth factors was examined with immortalized esophageal cells of mice (Katayama and Kan 1991), where we found that non-malignant esophageal cells required heparin-binding growth factors for growth. Malignant counterparts probably produce these factors through an autocrine mechanism. Insulin, transferrin, bovine serum albumin and fibronectin were necessary for the serum-free culture of esophageal cells. As in the case of epidermal keratinocytes, terminal differentiation was prevented by a low calcium concentration in the medium.

Cultured normal human esophageal cells have been used as a counterpart of cancer cell lines in the studies of esophageal cancer (Grace et al. 1985; Banks-Schlegel and Quintero 1986 a, b) and carcinogenesis (Banks-Schlegel et al. 1985; Cheng and Li 1985; Burg-Kurland et al. 1986; Sasajima et al. 1987). Their growth and differentiation have also been investigated (Banks-Schlegel et al. 1985, 1986 a; Katayama et al. 1986; Sasjima et al. 1987). Similar studies on immortal cell lines of normal rat esophageal epithelial cells have also been reported (Stoner et al. 1985, 1989; Babcock et al. 1983; Rearick et al. 1988).

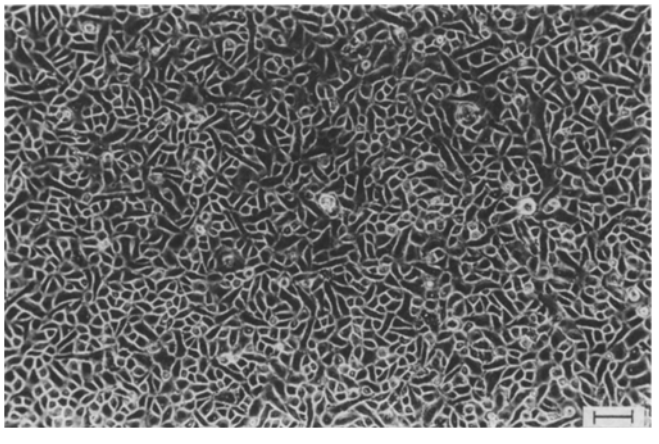

Fig. 1. Primary culture of normal human esophageal epithelial cells. *Bar*=50 μm

Cell lines derived from esophageal cancer

Table 1 lists cell lines derived from esophageal cancer. In general, they have been established in countries marked by higher incidences of esophageal cancers, namely, China, South Africa, and Japan. Of the 73 cell lines reported, 41 were established in Japan. These cell lines have been used for specific areas of research, according to the interests of researchers, such as hormone responses, hyperthermia chemotherapy and oncogenes.

TE-series cell lines (Fig. 2) were among the first to be established (Nishihira et al. 1979, 1984, 1985; Kuriya et al. 1983) and have been widely utilized within Japan and internationally. To date, 15 cell lines have been established from a total of 70 attempts, the success rate being 20%. As summarized in Table 2, all cultures but 2 were derived from primary lesions. Histologically, primary tumors were diagnosed as squamous cell carcinoma with varying degrees of differentiation, except for TE-7 which was derived from adenocarci-

Table 1. Cell lines established from esophageal cancer

Cell lines	No. of cell lines	References
TE series	15	Nishihira et al. 1979
KYSE series	21	Shimada et al. 1991
KSE series	2	Matsuoka et al. 1989, 1991
SGF series	2	Saito et al. 1987
EC-GI	1	Sato et al. 1987
ECa, EL series	7	Pan 1989
CE series	3	Hu et al. 1984
EC/CUHKI	1	Mok et al. 1987
HCU series	15	Robinson and Maistry 1983 Robinson 1986
SNO	1	Bey et al. 1976
HCE series	8	Banks-Schlegel 1985; Banks-Schlegel and Quintero 1986 a

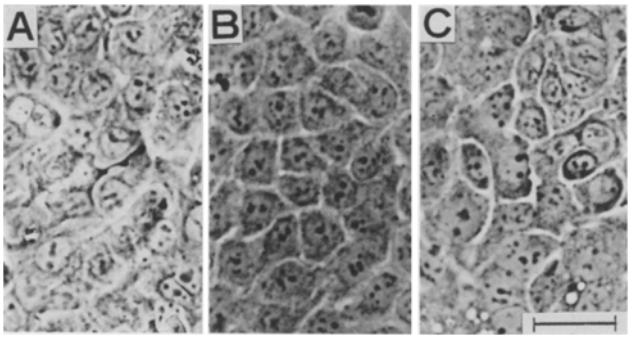

Fig. 2. TE-series cell lines: **A** TE-1; **B** TE-2; **C** TE-3. *Bar* = 50 μm

Table 2. Origin and some characteristics of TE-series esophageal cancer cells [a]

Cell line	Patient		Primary tumor			Culture material	Tumorigenicity in nude mice	Amplification and overexpression of gene
	Age	Sex	Histol	Stage	Diff			
TE-1	58	M	SCC	II	Well	Primary lesion	+	Amp, c-*erb*B; Over, EGF-R Mut, K-*ras*; Over, EGF+TGFα
TE-2	56	M	SCC	IV	Poor	Primary lesion	+	Mut, K-*ras*; Over, EGF-R+TGFα
TE-3	48	M	SCC	IV	Well	Subcutaneous lymph node metastasis	+	Amp, c-*erb*B
TE-4	48	F	SCC	III	Well	Primary lesion	–	
TE-5	73	F	SCC	IV	Poor	Primary lesion	–	Over, TGFα
TE-6	71	M	SCC	IV	Well	Primary lesion	+	Co-amp, *int*-2+*hst*-1
TE-7	72	M	Adeno	II		Primary lesion	–	Amp, c-*erb*B-2; Over, EGF-R, TGFα
TE-8	63	M	SCC	III	Mod	Primary lesion	–	Amp, c-*erb*B; Over, TGFα+EGF-R; Mut, H-*ras*; Co-amp, *int*-2+*hst*-1
TE-9	48	M	SCC	IV	Poor	Pleural effusion	–	Co-amp, *int*-2+*hst*-1
TE-10	58	M	SCC	IV	Well	Primary lesion	–	Co-amp, *int*-2+*hst*-1
TE-11	58	M	SCC	IV	Mod	Primary lesion	–	Co-amp, *int*-2+*hst*-1
TE-12	54	M	SCC	III	Mod	Primary lesion	–	Over, EGF-R+TGFα
TE-13	65	F	SCC	IV	Poor	Primary lesion	+	
TE-14	57	M	SCC	IV	Mod	Primary lesion	+	
TE-15	58	F	SCC	IV	Well	Primary lesion	+	

[a] SCC, squamous cell carcinoma: Adeno, adenocarcinoma; Diff, differentiation; Mod, moderate; Amp, amplification; Co-amp, co-amplification; Over, overexpression; Mut, mutation

noma. None of these tumors had been X-ray irradiated prior to surgery. These cells were grown in a monolayer in RPMI-1640 medium supplemented with 10% fetal calf serum. On heterotransplantation into nude mice, 7 cell lines produced tumors with a histological appearance similar to that of the original tumors. Cell lines were also established from these heterotransplanted tumors.

Growth and differentiation properties

Growth characteristics in tissue culture may reflect the malignant potential of primary tumors from which the cells were derived. Robinson et al. (1980) found that adaptation to in vitro culture conditions correlated well with invasiveness in vivo and prognosis at 6 months after surgery.

Like most cancer cells, esophageal cancer cells show a polygonal shape with varying morphology and tend to stratify in a disorganized fashion, yielding a higher saturation density than their normal counterparts. They grow anchorage-independently in soft agar medium though their efficiencies and sizes vary among cell lines (Banks-Schlegel and Quintero 1986 a). The use of lethally irradiated 3T3 cells as a feeder layer facilitates the growth of esophageal cancer cells (Matsuoka et al. 1991; Banks-Schlegel and Quintero 1986 a).

Alteration of cell-surface glycoproteins is known to be associated with malignant cell transformation. Indeed, the N-linked sugar chain was found to be altered in primary tumors of esophageal cancer (Hiraizumi et al. 1990). Takano et al. (1990 a) found that TE cells in the outer surface of colonies in a collagen gel matrix exhibited high affinity for binding to leukoagglutinin (L-PHA). The increase of L-PHA-reactive oligosaccharides may contribute to their malignant potential, possibly escaping macrophage-mediated surveillance (Takano et al. 1990 b).

Most esophageal cancer cell lines were derived from squamous cell carcinoma with a reduced degree of differentiation. Banks-Schlegel and Quintero (1986 a) found that differentiation features of keratinocytes are altered or reduced in esophageal cancer cells. Keratin proteins extracted from the HCE-series cell lines revealed altered profiles. Expression of 52-kDa keratin was reduced in most primary esophageal tumors and also in tumors in nude mice, but only in some cell lines. Reduced expression of 42-kDa and 52-kDa keratins coincides with the appearance of 67-kDa keratin, reflecting differentiation stages of these esophageal cancer cells. Terminal differentiation of keratinocytes can be measured by the formation of cornified envelopes. When differentiation was induced by a calcium ionophore, esophageal cancer cells often exhibited a reduced capacity to form a cornified envelope, ranging from 1% to 30% in cancer cells but from 70% to 90% in normal cells, suggesting the existence of a defect in the pathway of terminal differentiation.

Chromosomal aberrations

Analysis of chromosomes of cancer cells provides important information on possible genetic alterations underlying the development of cancer. However, the preparation of chromosomal specimens of solid tumors has drawbacks, and only limited knowledge is available in comparison with leukemia.

Detailed analyses of TE-, HCE-, HCU-series and Chinese esophageal cancer cells were reported by Su et al. (1988 a, b) and Whang-Peng et al. (1990). Esophageal cancer cells, like other types of cancer, have aneuploid chromosome numbers which vary according to cell lines. The chromosomes most frequently involved in structural abnormalities are chromosomes 1,3, 9 and 11. Chromosomal aberrations in TE-series cells include del(3) (:p11q22:) for TE-1, inv(3) (p21p24) for

TE-2, and del(3) (p11) and del(3) (:p13q13:) for TE-3 (Whang-Peng et al. 1990). Since rapid progress is being made in chromosomal gene mapping, the significance of these aberrations will eventually be elucidated.

Protein kinase C

Protein kinase C (PKC) plays a crucial role in signal transduction in the cell membrane mediated by hormones, growth factors, neurotransmitters and also tumor promoters. Chida et al. (1988) screened 41 cell lines for PKC activity and found that cancer cells show much lower activity in general than their normal counterparts: human epidermal keratinocytes in primary culture have an activity of 20.0 mU/10^6 cells, whereas the activities of TE-1, -2, -8 and -9 are 7.6, 1.4, 0.5, and 3.4 mU/10^6 cells respectively. This may be due to the increased turnover of phosphatidylinositol in cancer cells, resulting in their activation and subsequently in down-regulation.

We measured PKC activities of primary esophageal tumors and their adjacent normal mucosa (Hashimoto et al. 1989). Although considerable variations were found, PKC activities of the eight esophageal cancers were similar to those of the adjacent normal mucosa: the average PKC activities of the tumor tissues and normal mucosa were 7.5 and 8.3 pmol min^{-1} mg $protein^{-1}$, respectively, in their membrane fractions, and 7.9 and 7.8 pmol min^{-1} mg $protein^{-1}$ respectively, in their cytosolic fractions.

Molecular cloning studies have indicated that PKC molecules consist of a protein family that can be classified into three groups, Ca^{2+}-dependent conventional PKC (cPKCα, cPKCβI, cPKCβII and cPKCμ), Ca^{2+}-independent novel PKC (nPKC) and phorbol-ester-independent atypical PCK (aPKCγ and aPKCλ) (Nishizuka 1992). Although two isoforms (nPKCδ and nPKCε) were previously known for nPKC, we cloned two new members, termed nPKCη and nPKCθ from a cDNA library of mouse skin (Osada et al. 1990, 1992). By systematic survey, we found that nPKCη is the major PKC isoform expressed in epithelial tissue including that of the esophagus (Osada et al. 1993), implying a possible significant role of nPKCη in growth, differentiation and carcinogenesis of esophageal epithelium.

Growth factors and their receptors

EGF and TGFα. Growth of cells is regulated by growth factors, which are supplied by distant tissues through the endocrine mechanism, by neighboring cells through the paracrine mechanism, or by themselves through the autocrine mechanism. The autocrine loop of growth regulation is thought to be important for the growth of cancer cells. Yoshida et al. (1990) found that this is also the case with the TE-series cells. Expression of mRNA for transforming growth factor α (TGFα) was very high in all the 6 TE cell lines examined, while mRNA for epidermal growth factor (EGF) was expressed in 3 of 6 lines. Furthermore, antibodies against EGF and TGFα were found to inhibit DNA synthesis of TE-1 cells, which express both EGF and TGFα at high levels. These data suggest that TGFα and/or EGF act as an autocrine growth factor in esophageal cancer cells.

Although EGF stimulates growth of a wide variety of cells, we found that growth of squamous cell carcinomas, including TE-series cells, is inhibited by exogenously added EGF in a dose-dependent fashion (Kamata et al. 1986). The sensitivity to this inhibitory effect of EGF was found to correlate well with the elevated levels of EGF receptor.

Overexpression of EGF receptor. EGF and TGFα share EGF receptor, a product of the c-*erb*B proto-oncogene. Most normal cells, both of mesenchymal and epithelial origin, express EFG receptor at a level of 10^5/cell. Squamous cell carcinomas in cell culture have been reported to express a large amount of EGF receptor, up to 50-fold that of normal keratinocytes. We examined the EGF receptor gene and its expression in TE-series cells at the DNA, RNA and protein levels. Amplification and overexpression of this gene were found in these cells (Yamamoto et al. 1986; Kamata et al. 1986). TE-8 cells were found to amplify the c-*erb*B gene to an extent similar to that of A431 cells, which are known for amplification, expression of a higher amount of mRNA and the existence of binding sites with low affinity. In contrasting observations, however, Banks-Schlegel and Quintero (1986 b) reported that TE-, ECU- and HCE-series esophageal cancer cells contained lowered quantities of EGF receptor.

In the primary tumors of the high-risk regions, amplification of the c-*erb*B gene was also found in 5 of 37 samples from Linxian, China (Lu et al. 1988) and 2 of 25 samples from Normandy, France (Hollstein et al. 1988).

Thus, data suggest that changes in the gene copy number and/or high levels of expression of c-*erb*B may play an important role in the pathogenesis of esophageal cancer.

c-erbB-2. The c-*erb*B-2 gene encodes a protein that closely resembles EGF receptor. It is expressed in germinal epithelia, but not in adult epithelia. In human cancer, adenocarcinomas of the breast and stomach were found to amplify the c-*erb*B-2 gene (Yokota et al. 1986; Park et al. 1989). In keeping with this observation, we found that c-*erb*B-2 is amplified only in TE-6 derived from squamous cell carcinoma of the esophagus but not in the other 8 TE cell lines derived from squamous cell carcinoma (unpublished data). In primary esophageal cancer, however, Hollstein et al. (1988) reported no amplification of the c-*erb*B-2 gene in the 26 squamous cell carcinomas examined.

Oncogenes and tumor-suppressor genes

Absence of mutated ras *genes.* Studies to date suggest that oncogenes and tumor-suppressor genes are involved in the development of human malignancies to various degrees and in various combinations depending on the type of tumors. Among these, the *ras* oncogene family appears to play a prominent role; activated *ras* genes have been detected in most types of human cancers with various frequencies, e.g., 40% in colon cancer (Bos et al. 1987; Forrester et al. 1987) and 95% in pancreas cancer (Almoguera et al. 1988). Mutagenic activation of the *ras* oncogene family in human tumors is due to base substitutions in codon 12, 13 or 61.

Esophageal cancer seems to be an exception with regard to the activation of *ras* oncogenes. In a total of 93 primary tumors from high-risk regions of China (Jiang et al. 1989), Uruguay (Hollstein et al. 1991 a), South Africa (Victor et al.

1990) and France (Hollstein et al. 1988, 1991 a), none showed evidence of mutations at the "hot" codons of the *ras* oncogenes by polymerase chain reaction (PCR) techniques. Furthermore, no mutations of the *ras* genes were detected in 12 specimens obtained from Barrett's esophagus, a dysplastic lesion of the esophagus with a high risk of cancer (Meltzer et al.1990). We also found that there were no point mutations of H-*ras* and K-*ras* genes in 30 fresh surgical specimens examined. In the TE series, however, 3 out of 7 cell lines examined contained point mutations: at codon 12 of H-*ras* in TE-8 and of K-*ras* in TE-1 and -2 (Yamasaki et al. 1992, personal communication). Further studies are needed to determine whether this occasional mutation is characteristic of cultured cell lines or related to unknown etiological factors.

Co-amplification of hst-*1 and* int-*2*. Another unique feature of oncogenes in esophageal cancer is co-amplification of the *hst*-1 and *int*-2 genes. Such co-amplification was also observed in 5 of 13 TE cells examined (Katoh et al., personal communication). Wagata et al. (1991) reported amplification of the *int*-2 gene in 12 of 31 primary esophageal cancers. However, these genes were not expressed at the mRNA level (Tsuda et al. 1989), suggesting that an unidentified gene(s) located at this locus is amplified and expressed.

The *hst*-1 protein is a novel growth factor, being 40%–50% homologous to acidic and basic fibroblast growth factor (FGF), and the *int*-2 protein comprises a heparin-binding growth factor (HBGF) family. Receptors for the HBGF family include the N-*sam* and K-*sam* genes (Hattori et al. 1990, 1992). Katoh (personal communication) demonstrated expression of mRNA of K-*sam*, N-*sam* and basic FGF, but not acidic FGF, in all the 13 TE-series cell lines examined. However, no amplification or gross rearrangement of these genes was detected by Southern blot analysis. These results suggest that basic FGF plays an important role in the development of esophageal cancer in an autocrine or paracrine manner.

Amplification of the myc *gene*. Amplification of the c-*myc* gene was detected in 3 of 22 tumor DNA samples of esophageal cancers from Linxian, China (Lu et al. 1988). Interestingly, a rather high frequency (18 of 78) of amplification was observed in adjacent non-tumorous samples, possibly because of inclusion of hyperplasia, dysplasia or carcinoma in situ. In the TE-series cells, we found obvious amplification of the c-*myc* gene in 6 of 11 cell lines examined (unpublished data).

Mutations of the p53 gene. It is becoming apparent that the p53 tumor suppressor gene is involved in the development of many human cancers. The p53 gene is most probably a broad and important target for DNA damage in human carcinogenesis. Recent work suggests that mutations in relatively wide coding regions (i.e., exons 5–9) compromise its proper function of growth control.

Evidence for the involvement of p53 in esophageal cancer is now accumulating. Wagata et al. (1991) detected allelic loss of chromosome 17p, where the p53 gene is located, with high frequency (10 of 22 tumors) in primary esophageal cancer from the Kyoto region of Japan, while the frequencies of losses of other chromosomes were found to be lower. By the use of PCR, Meltzer et al. (1991) found loss of heterozygosity affecting the p53 gene in 14 of 27 primary esophageal cancers. In Barrett adenocarcinoma of the esophagus, Blount et al. (1991) found allelic loss of chromosome 17p in 12 of 13 tumors, of which 8 showed overexpression of p53 proteins.

These allelic losses are most likely due to mutations in the p53 gene. Hollstein et al. (1990, 1991 a) demonstrated the presence of p53 mutations in esophageal cancers from Lyon, France (5 of 14, 35.7%), Normandy, France (9 of 15, 60%), and Uruguay (6 of 19, 31.6%). Mutation of p53 was also observed in 2 of 4 HCE cell lines (Hollstein et al. 1990). All these mutations were dispersed over exons 5–9, most being missense mutations. Bennett et al. (1991) analyzed paraffin-embedded esophageal cancers from China for genetic and protein alterations of the p53 gene: more than half of the samples contained elevated p53 protein levels, most of which revealed missense mutations by PCR analysis. In Barrett epithelium adjacent to esophageal carcinoma, mutations, all localized to exon 5, were detected at a high frequency (4/7) by single-strand conformational-polymorphism analysis (Casson et al. 1991).

Hollstein et al. (1991 b) summarized the location and type of mutations in the p53 gene in various human cancers including esophageal cancer. They found transversions to be exceptionally frequent among esophageal cancers in comparison with the base-substitution patterns of most other cancers. These transversions occurred with similar frequency at G • C and A •T pairs, whereas in other solid tumors, changes at A • T pairs were uncommon.

Allelic loss of Rb, APC and MCC genes. Besides the p53 gene, several tumor-suppressor genes have been identified, cloned and sequenced. These include Rb from retinoblastoma and APC and MCC from familial adenomatous polyposis coli. Meltzer and his colleagues recently reported allelic loss at the loci of Rb, APC and MCC in primary esophageal cancer (Boynton et al. 1991, 1992). Using PCR, loss of heterozygosity of APC or MCC or both was detected in 20 of 26 (77%) informative cases. Similarly, allelic loss of the Rb gene was found in 19 of 40 (47.5%) informative tumors. Their frequencies in squamous cell carcinoma and Barrett adenocarcinoma were similar. These data suggest that inactivations of Rb, APC and/or MCC tumor-suppressor genes along with that of p53 are involved in the etiology and/or progression of esophageal cancer.

Alkylated DNA adducts

N-Nitrosamines are considered to be a major risk factor of human cancers, especially those of the gastrointestinal tract, including esophageal cancer. These alkylating nitrosamines result in alkylation at the N or O atom of the DNA bases, e.g., O^6-methyldeoxyguanosine (O^6-MedG). The presence of these DNA adducts was reported in surgical specimens of esophageal mucosa from patients at different risks of the cancer (Wild and Montesano 1991; Montesano et al. 1990; Umbenhauer et al. 1985). In Linxian, China, 18 out of 26 samples showed a high level of O^6-MedG ranging from 50 fmol/mg DNA to 161 fmol/mg DNA. In Normandy, France, the prevalence was also high (4/5), whereas it was low (1/11) in Rhone-Alpes, France, a low-risk region.

O^6-MedG causes mispairing with thymidine. Horsfall and Glickman (1988) reported that an esophageal carcinogen, *N*-nitroso-*N*-methyl-*N*-acetoxybenzylamine, produced G→A

transitions in the *lac*I gene of *E. coli*. Because G→A transition was seen most frequently (16 of 37 total mutations) in the p53 gene, nitrosamines are most likely implicated in causing the p53 mutation.

Therapy-oriented studies

Poor prognosis of esophageal cancer can be overcome by application of adequate and strong chemotherapy and/or radiation therapy before or after surgery. We reported elsewhere that postoperative chemo- or radiation therapy greatly improved survival rates: the 5-year survival rate for esophageal cancer patients with regional lymph node metastasis was 37% with such a combined therapy but only 15% with surgical treatment alone (Nishihira et al. 1984). Studies of the fundamental problems of these therapies are indispensable for improving the prognosis.

We investigated sensitivities of a series of TE cell lines to chemotherapeutic drugs such as mitomycin C, Adriamycin, bleomycin, 5-fluorouracil and cisplatinum, which are currently used for chemotherapy of solid tumors (Nishihira et al. 1985). Each TE cell line was found to be sensitive to certain drugs but resistant to others. Their responses to drugs seem to be "individual" and no trends or rules exist. Similar variability was also found with heterotransplanted tumors (Nishihira et al. 1985). Selection of effective drugs for each individual case of cancer, by the use of primary culture of tumors, is most desirable though difficulties may be encountered in practice.

Radiation sensitivity of esophageal cancer was examined by the use of explant-outgrowth culture (Mothersill et al. 1988). Cancer cells were found to be highly resistant relative to normal tissue.

Hyperthermia has been used for therapeutic treatment of solid tumors including esophageal cancer. Matsuoka et al. (1989) evaluated the combined application of hyperthermia, chemotherapy and X-irradiation by the use of KSE-1 cells. They found that hyperthermia, at a temperature of 42.5° C or more, significantly decreased growth of the cells and that a maximum effect was obtained when this treatment was combined with chemotherapy and X-irradiation. Saito et al. (1990) also found that esophageal cancer cells (SGF-3 and -5) were sensitive to hyperthermia at 42° C for 72 h.

Hypercalcemia is often associated with malignancies and results in various syndromes. Sato et al. (1987, 1988) established a cell line (EC-GI) from a patient with esophageal cancer accompanied by hypercalcemia. Heterotransplantation of this cell line caused marked hypercalcemia and bone resorption in nude mice. Analysis of the culture medium revealed that interleukin-1 and a parathyroid-hormone-like factor produced by these cells synergistically stimulate bone resorption, resulting in hypercalcemia.

Concluding remarks

More than 70 cell lines were established from esophageal cancer from the late 1970s to the late 1980s. With these cell lines, molecular and cellular features of esophageal cancer cells have been studied extensively. In addition to these cell lines, primary tumors have been used recently for analysis with molecular probes and antibodies. Data are now accumulating on molecular and cellular characteristics of esophageal cancer cells both in culture and in vivo, as summarized in Table 3.

One of the most significant features of esophageal cancer is the absence of mutation in *ras* genes among nearly 100 primary tumors examined from various high-risk regions, although other cancers of the gastrointestinal tract showed mutations of *ras* genes in various degrees.

Among a number of molecular changes observed in esophageal cancer in vivo and in vitro, amplification of the c-*erb*B, *hst* and *int* genes was commonly found. Because the amplification of *hst*-1 and *int*-2 was not accompanied by the overexpression of their messages or products, it is assumed that a gene(s) in the region of *hst*-1 and *int*-2 may play an important role in the etiology of esophageal cancer.

Another molecular feature of esophageal cancer is the mutation of the p53 tumor-suppressor gene, which was found

Table 3. Summary of changes in oncogenes, tumor-suppressor genes and their related genes in esophageal cancer

Gene	Primary tumors	Cell lines
ras oncogenes	No mutation (0/93)	Mutation of codon 12 of H- or K-*ras* (3/7 TE cells)
myc gene	Amplification (3/22 tumors, 18/78 adjacent mucosa)	Amplification (6/11 TE cells)
hst/*int*-2	Amplification of *int*-2 (12/31) Co-amplification of *hst* and *int*-2 (16/34), but no expression of *hst* or *int*-2 at mRNA	Co-amplification (5/13 TE cells)
bFGF	No data	Expression (13/13 TE cells)
Sam	No data	Expression (13/13 TE cells)
c-*erb*B	Amplification (7/62)	Amplification and overexpression in TE cells No amplification
c-*erb*B-2	No amplification (0/25)	Amplification in TE-7 (adenocarcinoma but not in other 9 TE cells Overexpression (6/9 TE cells)
EGF	No data	Overexpression (3/6 TE cells)
TGFα	No data	Overexpression (6/6 TE cells)
p53	Allelic loss of 17p (about 50%) Mutations (about 50%)	Mutation (2/4, HCE cells)
Rb	Allelic loss (19/40)	No data
APC/MCC	Allelic loss (20/26)	No data

in about 50% of cell lines and primary tumors of the esophagus. Mutations were dispersed in the region of exons 5–9. However, Barrett's epithelium adjacent to the tumors seems to be clustered in exon 5 (Casson et al. 1991). In esophageal cancers collected from high-risk regions including China, France and Uruguay, no association was found between geographical distribution and "hot spots" for the mutation. In liver cancers, however, the preferential site of the mutation was found for specimens from China, where both aflatoxin B1 and hepatitis B virus are risk factors (Yeh et al. 1985). The possible association of etiology or geographical distribution with site or nature of mutation should be further carefully examined in esophageal cancers, which also show a distinct geographical distribution.

Esophageal cancer is the sixth most common cancer worldwide. Prognosis of this cancer is poor compared with other types of cancer of the gastrointestinal tract, i.e., stomach and colon cancers. Achievement of a breakthrough in the prevention, diagnosis and treatment of esophageal cancer will become possible with a better understanding of the molecular and cellular features of cancer cells.

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