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The Synergistic Effects Study of C-myc and C-erbB-2 in the Carcinogenesis of Gastric Carcinoma

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Abstract

Objective: To study the significance of c-myc and c-erbB-2 oncogene expression in gastric cancer. **Methods:** 81 gastric cancer specimens were detected for c-myc and c-erbB-2 oncogene amplification using non-radioactive *in situ* hybridization method. **Results:** The amplification rates for c-myc and c-erbB-2 were 67.9% and 50.6% respectively, and there were significant correlation in the amplification of these two genes ($\chi^2 = 7.26$, $P < 0.01$). **Conclusions:** The amplification of c-myc and c-erbB-2 may play an important role in gastric cancer development, and these two genes may have synergistic effect.

Keywords

c-myc, c-erbB-2, Synergistic Effects

1. Introduction

The c-myc gene, mapped on human chromosome 8q24, encodes the transcription factor c-myc protein, which heterodimerizes with a partner protein, Max, to regulate gene expression. C-myc positively affects cell cycle regulation, apoptosis and metabolism, and negatively affects cellular differentiation and cell adhesion. Ectopic expression of the c-myc oncoprotein prevents cell cycle arrest in response to growth-inhibitory signals, leading to uncontrolled cell proliferation. Moreover, c-myc activation in quiescent cells was sufficient to induce cell cycle

entry in the absence of growth factors [1]. Deregulated c-myc expression and signal pathway contribute to the neoplastic phenotype of deregulated growth, anchorage-independent growth, and glycolytic metabolism. C-myc overexpression was observed in many different malignancies such as breast cancer, ovarian cancer, gastric cancer, lung cancer, colorectal cancer and other cancers [2]-[9]. Proto-oncogene c-erbB-2, also called neu of HER-2, is located on human chromosome 17q21. It belongs to a subfamily of type I receptor tyrosine protein kinase that encodes a 185 kDa transmembrane growth factor glycoprotein (P185) which contains an extracellular ligand-binding domain and intracellular tyrosine kinase activity. The extracellular region was similar in structure to that of epidermal-growth-factor receptor [10]. Like epidermal growth factor, c-erbB-2 expression represents a high proliferative activity of tumor cells [11]. Overexpression was observed in a variety of tumors such as breast, ovarian, gastric, lung, prostate, colonic, and other cancers [12] [13] [14] [15] [16]. In this paper, we studied c-erbB-2 and c-myc expression profile in GC tissues using *in situ* hybridization, objective to investigate the synergistic effects of c-erbB-2, and c-myc in the carcinogenesis of gastric carcinoma.

2. Patients and Methods

2.1. Clinical Specimens

Totally 81 (53 males and 28 females) gastric carcinoma specimens were collected from Jan 2016 to Jun 2017 from the affiliated hospital of Taishan Medical University and the oncology institute of medical college of Wuhan University. Age of the patients was from 37 to 65 years old (average 58.6 years). There were 35 (43.2%) cases with carcinomas at gastric cardia, 17 (21.0%) cases at gastric body and 29 (35.8%) cases at antrum. According to Lauren classification, there were 33 (40.7%) cases with intestinal type carcinoma and 48 (59.3) cases with diffused type carcinoma. Thirty-five (43.2%) cases were with well-differentiated carcinoma and 46 (56.8%) were poorly differentiated carcinoma. In 11 (13.6%) cases cancer invaded superficial muscle layer and in the rest cancer invaded beyond deep muscle. 57 (70.4%) cases had lymph node metastases and the other 24 (29.6%) cases not. After surgical removal, the specimens were snap frozen in liquid nitrogen and cryo-sectioned at −26˚C, with thickness between 6 to 10 μm, which were mounted on aminopropyltriethoxysilane (APES, Sigma, St Louis, Ohio, USA) treated slides. Informed consent was signed according to the Declaration of Helsinki (1975). The research protocol was approved by the human ethics committee of Affiliated Hospital of Taishan Medical University.

2.2. *In Situ* Hybridization

The procedures were in accordance to a previous study with a few modifications [16]. H-C-myc probe was purchased from Sina-American Biotechnology Company (No. 007, Sanshan Road, National Hi-Tech Industry Development Zone, Luoyang, Henan Province, China), with the length of 1.4 kb (Exon 3). C-erbB-2

cDNA probe was a kind gift of Professor Yamamoto from Tokyo University, Japan. It was the product of c-erbB-2 cDNA degradation by endonucleases Dra Iand Sma I, with the length of 461bp. Non-radioactive digoxigenin-11-UTP labeling was performed using random priming method, with reagent kit from Boehringer mannheims (purchased from Sino-American Biotechnology Company, No. 007, Sanshan Road, National Hi-Tech Industry Development Zone, Luoyang, Henan Province, China). The probe concentration was 15 μg/ml and sensitivity was 0.1 pg DNA. After treatment with 0.1 N HCl, RNase A, protease K and 0.1% Glycin for 5 minutes, the slides were pre-hybridized for one hour at room temperature in pre-hybridization solution (4 × SSC, 50% formamide, 1× Denhardt solution, 0.5% PEG and 0.5 mg/ml ssDNA). After washing the hybridization solution (probe concentration 0.2 μg/ml) was added, the slides were sealed with cover glass and treated in formamide chamber at 95˚C for 10 - 15 min. Then the slides were hybridized overnight at 42˚C in a humid chamber, after which the cover glass was removed in 2 × SSC solutions, and alkaline phosphatase labeled anti-DIG antibody (1:500 dilution) was added and incubated at 60˚C for 2 h. After washing, 5-bromo-4-chloro-3-indoxyl phosphate disodium (BCIP)/nitroblu-tetrazolium (NBT) color development substrate was added and kept in the dark for color reaction, which was terminated by TE buffer washing when color reaction was sufficient. The slides were counter-stained with eosin, and sealed. Normal gastric tissues from the same specimens were treated in the same fashion as negative controls.

2.3. Slides Interpretation

When the slides were viewed under microscope (400×), dark brown-blue granules and particles could be found in positively stained cells, and the background was stained red by eosin. Positive and negative stained specimens were recorded respectively. Data on conventional pathology of the specimens were collected from routine pathological report based on gross pathology and hematoxylin and eosin (HE) stained tissue slides.

2.4. Statistical Analysis

Comparisons between c-myc and C-erbB-2 positive rates in different pathological subgroups were analyzed using Chi-square test, with $P < 0.05$ as statistical significance.

3. Results

3.1. C-myc and C-erbB-2 Expression in GC Tissues

Of the 81 GC specimens, 55 (67.9%) were found to have c-myc overexpression, which showed dark-brown granules in cancer tissue (**Figure 1**).

Of the 81 GC specimens, 41 (50.6%) were found to have c-erbB-2 overexpression, which showed dark-brown granules in cancer tissue (**Figure 2**).

The relationship of c-myc and c-erbB-2 overexpression and the pathologic

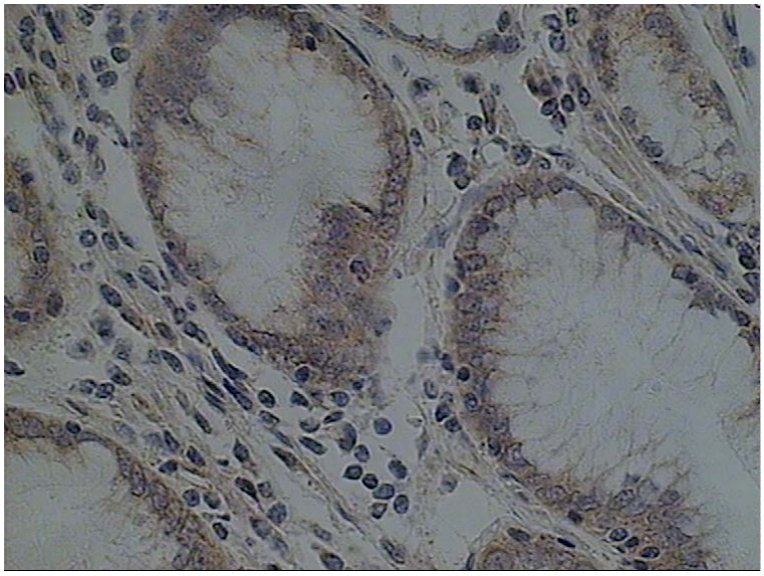

Figure 1. c-myc expression in gastric cancer tissues detected by non-radioactive labeled in situ hybridization. Frozen section gastric cancer slides were hybridized with digoxigenin-11-UTP labeled c-myc probe, developed by BCIP/NBT and counterstained by eosin. Positive stain was shown as dark brown particles in gastric cancer cell nests (Immunohistochemical staining: 400×).

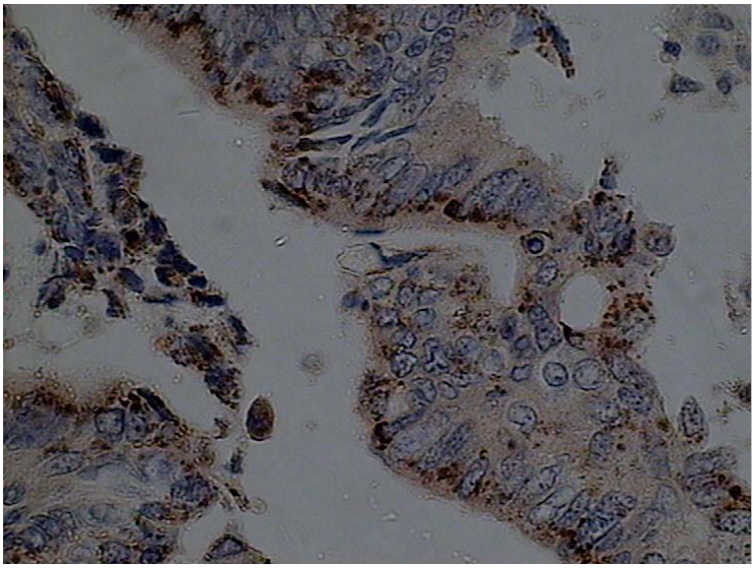

Figure 2. Non-radioactive labeled in situ hybridization to detect the expression of c-erbB-2 expression in gastric cancer tissues. Frozen section gastric cancer slides were hybridized with digoxigenin-11-UTP labeled c-erbB-2 probe, developed by BCIP/NBT and counterstained by eosin. Positive stain was shown as dark brown particles in gastric cancer cell nests (Immunohistochemical staining: 400×).

characteristics of tumors were summarized in **Table 1**.

3.2. Correlation between C-myc and C-erbB2 over Expression

The relationship between C-erbB2 over expression and C-myc over expression

Table 1. Relationship between the expression of c-myc and c-erbB-2 and pathological features in gastric cancer tissues n (%).

Pathological characteristics	Number	c-myc(+)	c-erbB-2(+)
Lauren classification			
Intestinal type	33	24 (72.7)	17 (51.5)
Diffuse type	48	31 (64.6)	24 (50.0)
Differentiation			
Well differentiated	35	26 (74.3)	12 (34.3)*
Poorly differentiated	46	29 (63.0)	29 (63.0)*
Depth of invasion			
Superficial muscle	11	9 (81.8)	2 (18.2)#
Deep muscle	70	46 (65.7)	39 (55.7)#
Lymph node metastases			
Yes	57	38 (66.7)	34 (59.6)**
No	24	17 (70.8)	7 (29.2)**

Compared to the two groups: *$\chi^2 = 5.48$, $P<0.05$; #$\chi^2 = 3.96$, $P < 0.05$; **$\chi^2 = 5.25$, $P < 0.05$

Table 2. Correlation analysis of c-erbB-2 and c-myc gene amplification in gastric cancer.

c-erbB-2	c-myc		Total
	+	-	
+	34	7	41
-	21	19	40
Total	55	26	81

$\chi^2 = 7.26$, $P < 0.01$

was studied in these 44 gastric carcinoma specimens and the results were presented in Table 2. Correlation analysis showed that the expression level of these two oncogenes in gastric cancer tissues were highly correlated ($\chi^2 = 7.26$, $P < 0.01$).

4. Discussion

Study on amplification of cancer gene in gastric cancer tissues, analyzed by Southern Houldsworth [17], 4 out of 28 cases of gastric carcinoma were c-erbB-2/HER-2, 1 case of only c-Met, the same method of analysis of 8 cases of gastric carcinoma with c-erbB-2 Mizutani [18], only 1 was amplified, other gene amplification has not formed sufficient information, and by immunohistochemical method of c-erbB-2 [16] has been found, c-erbB-3, c-myc, p21, p53 and other proteins have high expression in gastric cancer. The amplification of sensitive nonradioactive *in situ* hybridization detection of two kinds of cancer genes in gastric carcinoma, c-erbB-2 and c-myc were respectively 50.6% and 67.9%, indicating that both of the amplification were important biological factor in the

process of gastric cancer. Research shows that the metastasis of c-erbB-2 gene and gastric cancer tissue differentiation degree, invasion depth and lymph node ($P < 0.05$), degree of differentiation, depth of invasion was deeper, lymph node metastasis, c-erbB-2 gene expression rate was high, indicating activation of c-erbB-2 gene in cancer cells by play a role to make the depth of the stomach lymph node metastasis process, participate in the development and progression of gastric carcinoma. The c-myc oncogene was not related to the degree of differentiation, the depth of invasion and lymph node metastasis of gastric cancer.

It was also found that the c-erbB-2 and c-myc gene amplification was significantly correlated, which suggesting synergistic effect was generally believed that the synergistic effect of cancer gene was one of the important factors of cancer, c-erbB-2 was a growth factor receptor gene, susceptible to external factors to stimulate the mitogenic signal transduction to intracellular signal transduction the system, c-myc was a nuclear protein family of oncogenes, when signal transmission in the nucleus, c-myc amplification and expression, resulting in cell death. Our results confirm that this synergistic action was true in the pathogenesis of gastric cancer.

5. Conclusion

C-erbB-2 gene participates in the development and progression of gastric carcinoma. The c-myc oncogene was not related to the degree of differentiation, the depth of invasion and lymph node metastasis of gastric cancer. These two genes may have synergistic effect.

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Approval of the Ethics Committee

The study was performed according to the Declaration of Helsinki and was approved by the ethics committee of the affiliated hospital of Taishan University. Written informed consents were obtained from all the subjects recruited into our study in advance.

References

[1] Amati, B., Alevizopoulos, K. and Vlach, J. (1998) Myc and the Cell Cycle. *Frontiers in Bioscience*, **3**, D250-268. https://doi.org/10.2741/A239

[2] Knudsen, E.S., McClendon, A.K., Franco, J., Ertel, A., Fortina, P. and Witkiewicz, A.K. (2015) RB Loss Contributes to Aggressive Tumor Phenotypes in MYC-Driven Triple Negative Breast Cancer. *Cell Cycle*, **14**, 109-122. https://doi.org/10.4161/15384101.2014.967118

[3] Tang, B., Xie, R., Qin, Y., Xiao, Y.F., Yong, X., Zheng, L., Dong, H. and Yang, S.M. (2016) Human Telomerase Reverse Transcriptase (hTERT) Promotes Gastric Cancer Invasion through Cooperating with c-Myc to Upregulate Heparanase Expression. *Oncotarget*, **7**, 11364-11379. https://doi.org/10.18632/oncotarget.6575

[4] Ishii, H.H., Gobé, G.C., Pan, W., Yoneyama, J. and Ebihara, Y. (2002) Apoptosis and Cell Proliferation in the Development of Gastric Carcinomas: Associations with c-myc and p53 Protein Expression. *Journal of Gastroenterology and Hepatology*, **17**, 966-972. https://doi.org/10.1046/j.1440-1746.2002.02805.x

[5] Brägelmann, J., Böhm, S., Guthrie, M.R., Mollaoglu, G., Oliver, T.G. and Sos, M.L. (2017) Family Matters: How MYC Family Oncogenes Impact Small Cell Lung Cancer. *Cell Cycle*, **16**, 1489-1498. https://doi.org/10.1080/15384101.2017.1339849

[6] Xie, D., Sham, J.S., Zeng, W.F., Lin, H.L., Che, L.H., Wu, H.X., Wen, J.M., Fang, Y., Hu, L. and Guan, X.Y. (2003) Heterogeneous Expression and Association of Beta-Catenin, p16 and c-myc in Multistage Colorectal Tumorigenesis and Progression Detected By Tissue Microarray. *International Journal of Cancer*, **107**, 896-902. https://doi.org/10.1002/ijc.11514

[7] Li, F.L., Deng, H., Wang, H.W., Xu, R., Chen, J., Wang, Y.F., Li, X., Fan, B. and Li, B. (2011) Effects of External Application of Chinese Mcdicine on Diabetic Ulcers and the Expressions of β-Catenin,c-myc and K6. *Chinese Journal of Integrative Medicine*, **17**, 261-266. https://doi.org/10.1007/s11655-011-0703-5

[8] Albihn, A., Johnsen, J.I. and Henriksson, M.A. (2010) MYC in Oncogenesis and as a Target for Cancer Therapies. *Advances in Cancer Research*, **107**, 163-224. https://doi.org/10.1016/S0065-230X(10)07006-5

[9] Skvortsova, I., Skvortsov, S., Raju, U., Stasyk, T., Riesterer, O., Schottdorf, E.M., Popper, B.A., Schiestl, B., Eichberger, P., Debbage, P., Neher, A., Bonn, G.K., Huber, L.A., Milas, L. and Lukas, P. (2010) Epithelial-to-Mesenchymal Transition and c-myc Edpression Are the Detemninants of Cetuximab-Induced Enhancement of Squamous Cell Carcinoma Radioresponse. *Radiotherapy and Oncology*, **96**, 108-115. https://doi.org/10.1016/j.radonc.2010.04.017

[10] Lupu, R., Colomer, R., Kannan, B. and Lippman, M.E. (1992) Characterization of a Growth Factor that Binds Exclusively to the erbB-2 Receptor and Induces Cellular Responses. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 2287-2291. https://doi.org/10.1073/pnas.89.6.2287

[11] Lordick, F., Al-Batran, S.E., Dietel, M., Gaiser, T., Hofheinz, R.D., Kirchner, T., Kreipe, H.H., Lorenzen, S., Möhler, M., Quaas, A., Röcken, C., Rüschoff, J., Tannapfel, A., Thuss-Patience, P. and Baretton, G. (2017) HER2 Testing in Gastric Cancer: Results of a German Expert Meeting. *Journal of Cancer Research and Clinical Oncology*, **143**, 835-841. https://doi.org/10.1007/s00432-017-2374-x

[12] Hurvitz, S.A., Gelmon, K.A. and Tolaney, S.M. (2017) Optimal Management of Early and Advanced HER2 Breast Cancer. *American Society of Clinical Oncology Educational Book*, **37**, 76-92. https://doi.org/10.14694/EDBK_175630

[13] Bhaumik, S., Ahmad, F. and Das, B.R. (2016) Somatic Mutation Analysis of KRAS, BRAF, HER2 and PTEN in EGFR Mutation-Negative Non-Small Cell Lung Carcinoma: Determination of Frequency, Distribution Pattern and Identification of novel Deletion in HER2 Gene from Indian Patients. *Medical Oncology*, **33**, 117. https://doi.org/10.1007/s12032-016-0828-7

[14] Murray, N.P., Reyes, E., Fuentealba, C., Jacob, O. and Orellana, N. (2015) Possible Role of HER-2 in the Progression of Prostate Cancer from Primary Tumor to Androgen Independence. *Asian Pacific Journal of Cancer Prevention*, **16**, 6615-6619. https://doi.org/10.7314/APJCP.2015.16.15.6615

[15] Fanotto, V., Ongaro, E., Rihawi, K., Avallone, A., Silvestris, N., Fornaro, L., Vasile, E., Antonuzzo, L., Leone, F., Rosati, G., Giuliani, F., Bordonaro, R., Scartozzi, M., De Maglio, G., Negri, F.V., Fasola, G. and Aprile, G. (201) HER-2 Inhibition in Gastric and Colorectal Cancers: Tangible Achievements, Novel Acquisitions and Future Perspectives. *Oncotarget*, **7**, 69060-69074. https://doi.org/10.18632/oncotarget.11264

[16] Wang, K., Liu, J., Duan, Y., Wu, J., Dongye, S., Wang, Y., Liu, Z. and Han, G. (2017) C-erbB-2 Expression Is Related with Pathological Progression of Gastric Cancer: Results of a Non-Radioactive *in situ* Hybridization. *International Journal of Clinical and Experimental Pathology*, **10**, 9649-9653.

[17] Houldsworth, J., Cordon-Cardo, C., Ladanyi, M., Kelsen, D.P. and Chaganti, R.S. (1990) Gene Amplification in Gastric and Esophaged Adenocarcinomas. *Cancer Research*, **50**, 6417-6422.

[18] Mizutani, T., Onda, M. and Tokunaga, A. (1993) Relationship of c-erbB-2 Protein Expression and Gene Amplification to Invasion and Metastasis in Human Gastric Cancer. *Cancer*, **72**, 2083-2085. https://doi.org/10.1002/1097-0142(19931001)72:7<2083::AID-CNCR2820720705>3.0.CO;2-1