血漿 DNA 的定量與癌症的相關性

張玉芳、顏裕鴻、簡一治

E-mail: 9509689@mail.dyu.edu.tw

摘 要

癌症為基因變異所造成的疾病，它是由致癌基因、腫瘤抑制基 因及其他調節基因的變異，使調控細胞運作的許多機制發生改變， 讓正常細胞轉變為不受控制的腫瘤細胞。腫瘤細胞DNA 常因細胞 自殺或腫瘤壞死，將genomic DNA 釋放到血液中。惡性腫瘤從原 發腫瘤溢出，進入循環該處的血液或淋巴系統，將其傳送至體內其 它的組織，稱之為「轉移」，而轉移便是造成癌症死亡率增加的主 因。血漿DNA 在癌症的研究中，可能是個很好的指標。所以，在 本實驗我們利用即時定量PCR 進行比較正常人、肝癌、肺癌、初 發型以及轉移型乳癌患者血漿DNA 濃度之間的差異。在統計分析 結果上肝癌、肺癌、初發型以及轉移型乳癌患者與正常人血漿DNA 濃度皆有顯著差異(p21.427 則預後不佳。在本實驗中，我們提供較無侵襲性的方 法來檢測癌症病人血漿DNA 的遺傳變異，並且發現N-Myc 基因放 大現象的確可以當作預測乳癌發生的早期標誌。期許這樣較無侵入 性的檢驗方法在未來可以應用於癌症的臨床檢測。

關鍵詞 : 即時定量PCR，血漿DNA，致癌基因，癌症

目錄

參考文獻

1. Aarnio, M., Sankila, R., Pukkala, E., Salovaara, R., Aaltonen, L.A., de la Chapelle, A., Peltomaki, P., Mecklin, J.P. and Jarvinen, H.J. 1999. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer. 81: 214-218. 2. Anker, P., Lyautey, J., Lederrey, C. and Stroun, M. 2001. Circulating nucleic acids in plasma or serum. Clin Chim Acta. 313: 143-146. 3. Anker, P., Mulcahy, H., Chen, X.Q. and Stroun, M. 1999. Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. Cancer Metastasis Rev. 18: 65-73. 4. Anker, P. and Stroun, M. 2001. Tumor-related alterations in circulating DNA, potential for diagnosis, prognosis and detection of minimal residual disease. Leukemia. 15: 289-291. 5. Atkin, N.B. 2001. Microsatellite instability. Cytogenet Cell Genet. 92: 177-181. 6. Baselga, J. and Norton, L. 2002. Focus on breast cancer. Cancer Cell. 1: 319-322. 7. Bieche, I., Laurendeau, I., Tozlu, S., Olivi, M., Vidaud, D., Lidereau, R. and Vidaud, M. 1999. Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. Cancer Res. 59: 2759-2765. 8. Bieche, I., Parfait, B., Tozlu, S., Lidereau, R. and Vidaud, M. 2001. Quantitation of androgen receptor gene expression in sporadic breast tumors by real-time RT-PCR: evidence that MYC is an AR-regulated gene. Carcinogenesis. 22: 1521-1526. 9. Blancato, J., Singh, B., Liu, A., Liao, D.J. and Dickson, R.B. 2004. Correlation of amplification and overexpression of the c-myc oncogene in high-grade breast cancer: FISH, in situ hybridisation and immunohistochemical analyses. Br J Cancer. 90: 1612-1619. 10.Bockmann, B., Grill, H.J. and Giesing, M. 2001. Molecular characterization of minimal residual cancer cells in patients with solid tumors. Biomol Eng. 17: 95-111. 11.Bonnen, P.E., Wang, P.J., Kimmel, M., Chakraborty, R. and Nelson, D.L. 2002. Haplotype and linkage disequilibrium architecture for human cancer-associated genes. Genome Res. 12: 1846-1853. 12.Boynton, K.A., Summerhayes, I.C., Ahlquist, D.A. and Shuber, A.P. 2003. DNA integrity as a potential marker for stool-based

detection of colorectal cancer. Clin Chem. 49: 1058-1065. 13.Brodeur, G.M., Seeger, R.C., Schwab, M., Varmus, H.E. and Bishop, J.M. 1984. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science. 224: 1121-1124. 14.Castello, R., Estelles, A., Vazquez, C., Falco, C., Espana, F., Almenar, S.M., Fuster, C. and Aznar, J. 2002. Quantitative real-time reverse transcription-PCR assay for urokinase plasminogen activator, plasminogen activator inhibitor type 1, and tissue metalloproteinase inhibitor type 1 gene expressions in primary breast cancer. Clin Chem. 48: 1288-1295. 15.Chang, H.W., Ali, S.Z., Cho, S.K., Kurman, R.J. and Shih Ie, M. 2002a. Detection of allelic imbalance in ascitic supernatant by digital single nucleotide polymorphism analysis. Clin Cancer Res. 8: 2580-2585. 16.Chang, H.W., Lee, S.M., Goodman, S.N., Singer, G., Cho, S.K., Sokoll, L.J., Montz, F.J., Roden, R., Zhang, Z., Chan, D.W., Kurman, R.J. and Shih Ie, M. 2002b. Assessment of plasma DNA levels, allelic imbalance, and CA 125 as diagnostic tests for cancer. J Natl Cancer Inst. 94: 1697-1703. 17.Chen, X.Q., Stroun, M., Magnenat, J.L., Nicod, L.P., Kurt, A.M., Lyautey, J., Lederrey, C. and Anker, P. 1996. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med. 2: 1033-1035. 18.Claij, N. and te Riele, H. 1999. Microsatellite instability in human cancer: a prognostic marker for chemotherapy? Exp Cell Res. 246: 1-10. 19.Cohen, Y., Xing, M., Mambo, E., Guo, Z., Wu, G., Trink, B., Beller, U., Westra, W.H., Ladenson, P.W. and Sidransky, D. 2003. BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst. 95: 625-627. 20.Dang, C.V. 1999. c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol. 19: 1-11. 21.Davis, A.C., Wims, M., Spotts, G.D., Hann, S.R. and Bradley, A. 1993. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. Genes Dev. 7: 671-682. 22.de Alboran, I.M., O'Hagan, R.C., Gartner, F., Malynn, B., Davidson, L., Rickert, R., Rajewsky, K., DePinho, R.A. and Alt, F.W. 2001. Analysis of C-MYC function in normal cells via conditional gene-targeted mutation. Immunity. 14: 45-55. 23.Department of Health, T., R.O.C. 2002. Cancer registration system annual report. 24.Devilee, P., Cleton-Jansen, A.M. and Cornelisse, C.J. 2001. Ever since Knudson. Trends Genet. 17: 569-573. 25.Downs, K.M., Martin, G.R. and Bishop, J.M. 1989. Contrasting patterns of myc and N-myc expression during gastrulation of the mouse embryo. Genes Dev. 3: 860-869. 26.Driman, D., Thorner, P.S., Greenberg, M.L., Chilton-MacNeill, S. and Squire, J. 1994. MYCN gene amplification in rhabdomyosarcoma. Cancer. 73: 2231-2237. 27.Duesberg, P.H., Kawai, S., Wang, L.H., Vogt, P.K., Murphy, H.M. and Hanafusa, H. 1975. RNA of replication-defective strains of Rous sarcoma virus. Proc Natl Acad Sci U S A. 72: 1569-1573. 28.Felsher, D.W. and Bishop, J.M. 1999. Reversible tumorigenesis by MYC in hematopoietic lineages. Mol Cell. 4: 199-207. 29.Fields, C., Adams, M.D., White, O. and Venter, J.C. 1994. How many genes in the human genome? Nat Genet. 7: 345-346. 30.Freeman, W.M., Robertson, D.J. and Vrana, K.E. 2000. Fundamentals of DNA hybridization arrays for gene expression analysis. Biotechniques. 29: 1042-1046, 1048-1055. 31.Funa, K., Steinholtz, L., Nou, E. and Bergh, J. 1987. Increased expression of N-myc in human small cell lung cancer biopsies predicts lack of response to chemotherapy and poor prognosis. Am J Clin Pathol. 88: 216-220. 32.Gal, S., Fidler, C., Lo, Y.M., Taylor, M., Han, C., Moore, J., Harris, A.L. and Wainscoat, J.S. 2004. Quantitation of circulating DNA in the serum of breast cancer patients by real-time PCR. Br J Cancer. 90: 1211-1215. 33.Garson, J.A., McIntyre, P.G. and Kemshead, J.T. 1985. N-myc amplification in malignant astrocytoma. Lancet. 2: 718-719. 34.Gerner, E.W., Ignatenko, N.A., Lance, P. and Hurley, L.H. 2005. A comprehensive strategy to combat colon cancer targeting the adenomatous polyposis coli tumor suppressor gene. Ann N Y Acad Sci. 1059: 97-105. 35.Giacona, M.B., Ruben, G.C., Iczkowski, K.A., Roos, T.B., Porter, D.M. and Sorenson, G.D. 1998. Cell-free DNA in human blood plasma: length measurements in patients with pancreatic cancer and healthy controls. Pancreas. 17: 89-97. 36.Ginzinger, D.G. 2002. Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. Exp Hematol. 30: 503-512. 37.Grandori, C., Cowley, S.M., James, L.P. and Eisenman, R.N. 2000. The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol. 16: 653-699. 38.Grushko, T.A., Dignam, J.J., Das, S., Blackwood, A.M., Perou, C.M., Ridderstrale, K.K., Anderson, K.N., Wei, M.J., Adams, A.J., Hagos, F.G., Sveen, L., Lynch, H.T., Weber, B.L. and Olopade, O.I. 2004. MYC is amplified in BRCA1-associated breast cancers. Clin Cancer Res. 10: 499-507. 39.Hirning, U., Schmid, P., Schulz, W.A., Rettenberger, G. and Hameister, H. 1991. A comparative analysis of N-myc and c-myc expression and cellular proliferation in mouse organogenesis. Mech Dev. 33: 119-125. 40.Huang, Z.H., Li, L.H. and Hua, D. 2006. Quantitative analysis of plasma circulating DNA at diagnosis and during follow-up of breast cancer patients. Cancer Lett. 41.Hurlin, P.J. 2005. N-Myc functions in transcription and development. Birth Defects Res C Embryo Today. 75: 340-352. 42.Jahr, S., Hentze, H., Englisch, S., Hardt, D., Fackelmayer, F.O., Hesch, R.D. and Knippers, R. 2001. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 61: 1659-1665. 43.Jemal, A., Tiwari, R.C., Murray, T., Ghafoor, A., Samuels, A., Ward, E., Feuer, E.J. and Thun, M.J. 2004. Cancer statistics, 2004. CA Cancer J Clin. 54: 8-29. 44.Jiricny, J. 1996. Mismatch repair and cancer. Cancer Surv. 28: 47-68. 45.Key, T.J., Verkasalo, P.K. and Banks, E. 2001. Epidemiology of breast cancer. Lancet Oncol. 2: 133-140. 46.Kim, J.C., Roh, S.A., Koo, K.H., Ka, I.H., Kim, H.C., Yu, C.S., Lee, K.H., Kim, J.S., Lee, H.I. and Bodmer, W.F. 2004. Genotyping possible polymorphic variants of human mismatch repair genes in healthy Korean individuals and sporadic colorectal cancer patients. Fam Cancer. 3: 129-137. 47.Kim, K.H., Yoon, M.S., Na, Y.J., Park, C.S., Oh, M.R. and Moon, W.C. 2006a. Development and evaluation of a highly sensitive human papillomavirus genotyping DNA chip. Gynecol Oncol. 100: 38-43. 48.Kim, W.K., In, Y.J., Kim, J.H., Cho, H.J., Kim, J.H., Kang, S., Lee, C.Y. and Lee, S.C. 2006b. Quantitative relationship of dioxin-responsive gene expression to dioxin response element in Hep3B and HepG2 human hepatocarcinoma cell lines. Toxicol Lett. 49.Kinoshita, M., Nakamura, J., Kusaka, H., Hadama, T., Bago, K., Kitajima, M. and Baba, S. 1999. Automated and simultaneous identification of microsatellite instability by fluorescence-based polymerase chain reaction (PCR) in four loci. Clin Chim Acta. 279: 15-23. 50.Kinzler, K.W. and Vogelstein, B. 1996. Lessons from hereditary colorectal cancer. Cell. 87: 159-170. 51.Kohl, N.E., Kanda, N., Schreck, R.R., Bruns, G., Latt, S.A., Gilbert, F. and Alt, F.W. 1983. Transposition and amplification of oncogene-related sequences in

human neuroblastomas. Cell. 35: 359-367. 52.Koyama, M., Nagai, H., Bando, K., Matsumoto, S., Tajiri, T., Onda, M., Ito, M., Moriyama, Y. and Emi, M. 2000. New target region of allelic loss in hepatocellular carcinomas within a 1-cM interval on chromosome 6q23. J Hepatol. 33: 85-90. 53.Krieg, R.C., Paweletz, C.P., Liotta, L.A. and Petricoin, E.F., 3rd. 2002. Clinical proteomics for cancer biomarker discovery and therapeutic targeting. Technol Cancer Res Treat. 1: 263-272. 54.Kurzawski, G., Safranow, K., Suchy, J., Chlubek, D., Scott, R.J. and Lubinski, J. 2002. Mutation analysis of MLH1 and MSH2 genes performed by denaturing high-performance liquid chromatography. J Biochem Biophys Methods. 51: 89-100. 55.Lecomte, T., Berger, A., Zinzindohoue, F., Micard, S., Landi, B., Blons, H., Beaune, P., Cugnenc, P.H. and Laurent-Puig, P. 2002. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. Int J Cancer. 100: 542-548. 56.Lee, W.H., Murphree, A.L. and Benedict, W.F. 1984. Expression and amplification of the N-myc gene in primary retinoblastoma. Nature. 309: 458-460. 57.Levens, D. 2002. Disentangling the MYC web. Proc Natl Acad Sci U S A. 99: 5757-5759. 58.Loeb, L.A. 1994. Microsatellite instability: marker of a mutator phenotype in cancer. Cancer Res. 54: 5059-5063. 59.Lowe, S.W., Cepero, E. and Evan, G. 2004. Intrinsic tumour suppression. Nature. 432: 307-315. 60.Lu, X., Pearson, A. and Lunec, J. 2003. The MYCN oncoprotein as a drug development target. Cancer Lett. 197: 125-130. 61.Macgregor, P.F. and Squire, J.A. 2002. Application of microarrays to the analysis of gene expression in cancer. Clin Chem. 48: 1170-1177. 62.Mairal, A., Pinglier, E., Gilbert, E., Peter, M., Validire, P., Desjardins, L., Doz, F., Aurias, A. and Couturier, J. 2000. Detection of chromosome imbalances in retinoblastoma by parallel karyotype and CGH analyses. Genes Chromosomes Cancer. 28: 370-379. 63.Malynn, B.A., de Alboran, I.M., O'Hagan, R.C., Bronson, R., Davidson, L., DePinho, R.A. and Alt, F.W. 2000. N-myc can functionally replace c-myc in murine development, cellular growth, and differentiation. Genes Dev. 14: 1390-1399. 64.Mao, L., Schoenberg, M.P., Scicchitano, M., Erozan, Y.S., Merlo, A., Schwab, D. and Sidransky, D. 1996. Molecular detection of primary bladder cancer by microsatellite analysis. Science. 271: 659-662. 65.McAdams, H.H. and Shapiro, L. 1995. Circuit simulation of genetic networks. Science. 269: 650-656. 66.McPherson, K., Steel, C.M. and Dixon, J.M. 2000. ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. Bmj. 321: 624-628. 67.Merlo, A., Mabry, M., Gabrielson, E., Vollmer, R., Baylin, S.B. and Sidransky, D. 1994a. Frequent microsatellite instability in primary small cell lung cancer. Cancer Res. 54: 2098-2101. 68.Merlo, G.R., Venesio, T., Taverna, D., Marte, B.M., Callahan, R. and Hynes, N.E. 1994b. Growth suppression of normal mammary epithelial cells by wild-type p53. Oncogene. 9: 443-453. 69.Mizukami, Y., Nonomura, A., Takizawa, T., Noguchi, M., Michigishi, T., Nakamura, S. and Ishizaki, T. 1995. N-myc protein expression in human breast carcinoma: prognostic implications. Anticancer Res. 15: 2899-2905. 70.Mugrauer, G., Alt, F.W. and Ekblom, P. 1988. N-myc proto-oncogene expression during organogenesis in the developing mouse as revealed by in situ hybridization. J Cell Biol. 107: 1325-1335. 71.Nawroz, H., Koch, W., Anker, P., Stroun, M. and Sidransky, D. 1996. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med. 2: 1035-1037. 72.Nesbit, C.E., Tersak, J.M. and Prochownik, E.V. 1999. MYC oncogenes and human neoplastic disease. Oncogene. 18: 3004-3016. 73.Nisen, P.D., Zimmerman, K.A., Cotter, S.V., Gilbert, F. and Alt, F.W. 1986. Enhanced expression of the N-myc gene in Wilms' tumors. Cancer Res. 46: 6217-6222. 74.Pelengaris, S., Littlewood, T., Khan, M., Elia, G. and Evan, G. 1999. Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. Mol Cell. 3: 565-577. 75.Peltomaki, P., Gao, X. and Mecklin, J.P. 2001. Genotype and phenotype in hereditary nonpolyposis colon cancer: a study of families with different vs. shared predisposing mutations. Fam Cancer. 1: 9-15. 76.Pharoah, P.D., Day, N.E., Duffy, S., Easton, D.F. and Ponder, B.A. 1997. Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer. 71: 800-809. 77.Pritzker, K.P. 2002. Cancer biomarkers: easier said than done. Clin Chem. 48: 1147-1150. 78.Raggi, C.C., Bagnoni, M.L., Tonini, G.P., Maggi, M., Vona, G., Pinzani, P., Mazzocco, K., De Bernardi, B., Pazzagli, M. and Orlando, C. 1999. Real-time quantitative PCR for the measurement of MYCN amplification in human neuroblastoma with the TaqMan detection system. Clin Chem. 45: 1918-1924. 79.Robanus-Maandag, E.C., Bosch, C.A., Kristel, P.M., Hart, A.A., Faneyte, I.F., Nederlof, P.M., Peterse, J.L. and van de Vijver, M.J. 2003. Association of C-MYC amplification with progression from the in situ to the invasive stage in C-MYC-amplified breast carcinomas. J Pathol. 201: 75-82. 80.Ryan, K.M. and Birnie, G.D. 1996. Myc oncogenes: the enigmatic family. Biochem J. 314 (Pt 3): 713-721. 81.Savelyeva, L. and Schwab, M. 2001. Amplification of oncogenes revisited: from expression profiling to clinical application. Cancer Lett. 167: 115-123. 82.Sawai, S., Shimono, A., Hanaoka, K. and Kondoh, H. 1991. Embryonic lethality resulting from disruption of both N-myc alleles in mouse zygotes. New Biol. 3: 861-869. 83.Sawai, S., Shimono, A., Wakamatsu, Y., Palmes, C., Hanaoka, K. and Kondoh, H. 1993. Defects of embryonic organogenesis resulting from targeted disruption of the N-myc gene in the mouse. Development. 117: 1445-1455. 84.Schor, N.F. 1999. Neuroblastoma as a neurobiological disease. J Neurooncol. 41: 159-166. 85.Schwab, M., Ellison, J., Busch, M., Rosenau, W., Varmus, H.E. and Bishop, J.M. 1984. Enhanced expression of the human gene N-myc consequent to amplification of DNA may contribute to malignant progression of neuroblastoma. Proc Natl Acad Sci U S A. 81: 4940-4944. 86.Singer, G., Oldt, R., 3rd, Cohen, Y., Wang, B.G., Sidransky, D., Kurman, R.J. and Shih Ie, M. 2003. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst. 95: 484-486. 87.Sozzi, G., Conte, D., Leon, M., Ciricione, R., Roz, L., Ratcliffe, C., Roz, E., Cirenei, N., Bellomi, M., Pelosi, G., Pierotti, M.A. and Pastorino, U. 2003. Quantification of free circulating DNA as a diagnostic marker in lung cancer. J Clin Oncol. 21: 3902-3908. 88.Sozzi, G., Conte, D., Mariani, L., Lo Vullo, S., Roz, L., Lombardo, C., Pierotti, M.A. and Tavecchio, L. 2001. Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients. Cancer Res. 61: 4675-4678. 89.Srinivas, P.R., Kramer, B.S. and Srivastava, S. 2001a. Trends in biomarker research for cancer detection. Lancet Oncol. 2: 698-704. 90.Srinivas, P.R., Srivastava, S., Hanash, S. and Wright, G.L., Jr. 2001b. Proteomics in early detection of cancer. Clin Chem. 47: 1901-1911. 91.Starinsky, S., Figer, A., Ben-Asher, E., Geva, R., Flex, D., Fidder, H.H., Zidan, J., Lancet, D. and Friedman, E. 2005. Genotype phenotype correlations in Israeli colorectal cancer patients. Int J Cancer. 114: 58-73.

92.Sturgeon, C. 2002. Practice guidelines for tumor marker use in the clinic. Clin Chem. 48: 1151-1159. 93.Suzuki, N., Yoshida, A. and Nakano, Y. 2005. Quantitative analysis of multi-species oral biofilms by TaqMan Real-Time PCR. Clin Med Res. 3: 176-185. 94.Thiagalingam, S., Foy, R.L., Cheng, K.H., Lee, H.J., Thiagalingam, A. and Ponte, J.F. 2002. Loss of heterozygosity as a predictor to map tumor suppressor genes in cancer: molecular basis of its occurrence. Curr Opin Oncol. 14: 65-72. 95.Trumpp, A., Refaeli, Y., Oskarsson, T., Gasser, S., Murphy, M., Martin, G.R. and Bishop, J.M. 2001. c-Myc regulates mammalian body size by controlling cell number but not cell size. Nature. 414: 768-773. 96.Walker, W., Zhou, Z.Q., Ota, S., Wynshaw-Boris, A. and Hurlin, P.J. 2005. Mnt-Max to Myc-Max complex switching regulates cell cycle entry. J Cell Biol. 169: 405-413. 97.Wang, B.G., Huang, H.Y., Chen, Y.C., Bristow, R.E., Kassauei, K., Cheng, C.C., Roden, R., Sokoll, L.J., Chan, D.W. and Shih Ie, M. 2003a. Increased plasma DNA integrity in cancer patients. Cancer Res. 63: 3966-3968. 98.Wang, S.L., Cai, B., Cui, C.B., Liu, H.W., Wu, C.F. and Yao, X.S. 2003b.
[Apoptosis of human chronic myeloid leukemia k562 cell induced by prosapogenin B of dioscin (P.B) in vitro]. Ai Zheng. 22: 795-800. 99.Weeraratna, A.T. 2005. Discovering causes and cures for cancer from gene expression analysis. Ageing Res Rev. 4: 548-563. 100.Weiss, W.A., Aldape, K., Mohapatra, G., Feuerstein, B.G. and Bishop, J.M. 1997. Targeted expression of MYCN causes neuroblastoma in transgenic mice. Embo J. 16: 2985-2995. 101.Westermann, F. and Schwab, M. 2002. Genetic parameters of neuroblastomas. Cancer Lett. 184: 127-147. 102.Wijnhoven, S.W., Kool, H.J., van Teijlingen, C.M., van Zeeland, A.A. and Vrieling, H. 2001. Loss of heterozygosity in somatic cells of the mouse. An important step in cancer initiation? Mutat Res. 473: 23-36. 103.Wilhelm, J. and Pingoud, A. 2003. Real-time polymerase chain reaction. Chembiochem. 4: 1120-1128. 104.Wilhelm, J., Pingoud, A. and Hahn, M. 2003a. Real-time PCR-based method for the estimation of genome sizes. Nucleic Acids Res. 31: e56. 105.Wilhelm, J., Pingoud, A. and Hahn, M. 2003b. SoFAR: software for fully automatic evaluation of real-time PCR data. Biotechniques. 34: 324-332. 106.Wong, A.J., Ruppert, J.M., Eggleston, J., Hamilton, S.R., Baylin, S.B. and Vogelstein, B. 1986. Gene amplification of c-myc and N-myc in small cell carcinoma of the lung. Science. 233: 461-464. 107.Wu, C.L., Roz, L., Sloan, P., Read, A.P., Holland, S., Porter, S., Scully, C., Speight, P.M. and Thakker, N. 1997. Deletion mapping defines three discrete areas of allelic imbalance on chromosome arm 8p in oral and oropharyngeal squamous cell carcinomas. Genes Chromosomes Cancer. 20: 347-353. 108.Xu, L., Morgenbesser, S.D. and DePinho, R.A. 1991. Complex transcriptional regulation of myc family gene expression in the developing mouse brain and liver. Mol Cell Biol. 11: 6007-6015. 109.Zanella, I., Rossini, A., Domenighini, D., Albertini, A. and Cariani, E. 2002. Real-time quantitation of hepatitis B virus (HBV) DNA in tumorous and surrounding tissue from patients with hepatocellular carcinoma. J Med Virol. 68: 494-499. 110.Zimmerman, K. and Alt, F.W. 1990. Expression and function of myc family genes. Crit Rev Oncog. 2: 75-95. 111.Zimmerman, K.A., Yancopoulos, G.D., Collum, R.G., Smith, R.K., Kohl, N.E., Denis, K.A., Nau, M.M., Witte, O.N., Toran-Allerand, D., Gee, C.E. and et al. 1986. Differential expression of myc family genes during murine development. Nature. 319: 780-783.