

Furthermore, the number of examined cases was small, however, we initially observed cancer specific methylation not in *MRP-1* and *PMP-22*, but in *CASP-3*. Among caspase related genes, the genetic or epigenetic alterations of *CASP-3* have been reported until today, however, there has been no study about those of *CASP-3* which is located at the down-stream of caspase-8 and plays a central role in apoptosis signal network [32–34]. This result indicated that the methylation of *CASP-3* can be a carcinogen alone without involvement of other caspase related up-stream molecules in breast cancer cells. However, the population of the methylated case is small (16.7%), therefore, further studies are required to determine the main mechanism of the reduced expression.

In conclusion, we confirm that one can achieve expression profiling and evaluation by LMD, T7-based amplification and cDNA microarrays in breast cancer. Moreover, we have confirmed the role of *MRP-1* as an indicator of malignant potential in breast cancer cells specifically. In addition, we would use the present data of the methylation status of genes as an initial clue to the identification of critical metastasis related genes in further studies. Admittedly, however, we should keep in mind that the interaction between cancer cells and the adjacent normal cells can be very important in the understanding of practical cancer biology.

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References

- Mimori K, Matsuyama A, Yoshinaga K et al. Localization of thymidine phosphorylase gene in colorectal carcinoma tissues by *in situ* RT-PCR assay. *Oncology* 2002; 62: 327–32.
- Inoue H, Matsuyama A, Mimori K et al. Prognostic score of gastric cancer determined by cDNA microarray. *Clin Cancer Res* 2002; 8: 3475–9.
- Adeyinka A, Emberley E, Niu Y et al. Analysis of gene expression in ductal carcinoma in situ of the breast. *Clin Cancer Res* 2002; 8: 3788–95.
- Aoyagi K, Tatsuta T, Nishigaki M et al. A faithful method for PCR-mediated global mRNA amplification and its integration into microarray analysis on laser-captured cells. *Biochem Biophys Res Commun* 2003; 300: 915–20.
- Mori M, Mimori K, Yoshikawa Y et al. Analysis of the gene-expression profile regarding the progression of human gastric carcinoma. *Surgery* 2002; 131: S39–47.
- Mori M, Barnard GF, Mimori K et al. Overexpression of matrix metalloproteinase-7 mRNA in human colon carcinomas. *Cancer* 1995; 75: 1516–9.
- Honda M, Mori M, Ueo H et al. Matrix metalloproteinase-7 expression in gastric carcinoma. *Gut* 1996; 39: 444–8.
- Yamashita K, Mori M, Shiraishi T et al. Clinical significance of matrix metalloproteinase-7 expression in esophageal carcinoma. *Clin Cancer Res* 2000; 6: 1169–74.
- Sgroi DC, Teng S, Robinson G et al. *In vivo* gene expression profile analysis of human breast cancer progression. *Cancer Res* 1999; 59: 5656–61.
- Wulfkuhle J, Sgroi D, Krutzsch H et al. Proteomics of human breast ductal carcinoma *in situ*. *Cancer Res* 2002; 62: 6740–9.
- Mori M, Mimori K, Shiraishi T et al. Motility related protein 1 (*MRP1/CD9*) expression in colon cancer. *Clin Cancer Res* 1998; 4: 1507–10.
- Izumi Y, Hirata M, Hasuwa H et al. A metalloprotease-disintegrin, MDC9/meltrin-gamma/ADAM9 and PKCdelta are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *EMBO J* 1998; 17: 7260–72.
- Silvestrini R, Benini E, Veneroni S et al. p53 and bcl-2 expression correlates with clinical outcome in a series of node-positive breast cancer patients. *J Clin Oncol* 1996; 14: 1604–10.
- Sierra A, Castellsague X, Tortola S et al. Apoptosis loss and bcl-2 expression: Key determinants of lymph node metastases in T1 breast cancer. *Clin Cancer Res* 1996; 2: 1887–94.
- Sierra A, Castellsague X, Escobedo A et al. Bcl-2 with loss of apoptosis allows accumulation of genetic alterations: a pathway to metastatic progression in human breast cancer. *Int J Cancer* 2000; 89: 142–7.
- Kobayashi D, Yamada M, Kamagata C et al. Overexpression of early growth response-1 as a metastasis-regulatory factor in gastric cancer. *Anticancer Res* 2002; 22: 3963–70.
- Mhawech P, Herrmann F, Coassin M et al. Motility-related protein 1 (*MRP-1/CD9*) expression in urothelial bladder carcinoma and its relation to tumor recurrence and progression. *Cancer* 2003; 98: 1649–57.
- Mhawech P, Dulguerov P, Tschanz E et al. Motility-related protein-1 (*MRP-1/CD9*) expression can predict disease-free survival in patients with squamous cell carcinoma of the head and neck. *Br J Cancer* 2004; 90: 471–5.
- Dong J, Lamb P, Rinker-Schaeffer C et al. KAI1, a metastasis suppressor gene for prostate cancer on human chromosome 11p11.2. *Science* 1995; 268: 884–6.
- Si Z, Hersey P. Expression of the neuroendocrine antigen and analogues in melanoma CD9 expression appears inversely related to metastatic potential of melanoma. *Int J Cancer* 1993; 54: 37–43.
- Miyake M, Nakano K, Ieki Y et al. Motility related protein 1 (*MRP-1/CD9*) expression: Inverse correlation with metastases in breast cancer. *Cancer Res* 1995; 55: 4127–31.
- Miyake M, Nakano K, Itoi SI et al. Motility-related protein-1 (*MRP-1/CD9*) reduction as a factor of poor prognosis in breast cancer. *Cancer Res* 1996; 56: 1244–9.
- Hildebrandt T, van Dijk MC, van Muijen GN et al. Loss of heterozygosity of gene THW is frequently found in melanoma metastases. *Anticancer Res* 2001; 21: 1071–80.
- Widschwendter A, Tonko-Geymayer S, Welte T et al. Prognostic significance of signal transducer and activator of transcription 1 activation in breast cancer. *Clin Cancer Res* 2002; 8: 3065–74.
- Jeffrey IW, Bushell M, Tilleray VJ et al. Inhibition of protein synthesis in apoptosis: differential requirements by the tumor necrosis factor alpha family and a DNA-damaging agent for caspases and the double-stranded RNA-dependent protein kinase. *Cancer Res* 2002; 62: 2272–80.
- Megha T, Lazzi S, Ferrari F et al. Expression of the G2-M checkpoint regulators cyclin B1 and P34CDC2 in breast cancer: a correlation with cellular kinetics. *Anticancer Res* 1999; 19: 163–9.
- Tanaka K, Iwamoto S, Gon G et al. Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. *Clin Cancer Res* 2000; 6: 127–34.
- Bufalo D, Biroccio A, Leonetti C et al. Bcl-2 overexpression enhances the metastatic potential of a human breast cancer line. *FASEB J* 1997; 11: 947–53.
- Matsuyama A, Inoue H, Shibuta K et al. Hepatoma-derived growth factor is associated with reduced sensitivity to irradiation in esophageal cancer. *Cancer Res* 2001; 61: 5714–7.
- Balbin M, Pendas AM, Uria JA et al. Expression and regulation of collagenase-3 (*MMP-13*) in human malignant tumors. *Apmis* 1999; 107: 45–53.

31. Shuai K. Modulation of STAT signaling by STAT-interacting proteins. *Oncogene* 2000; 19: 2638-44.
32. Kim HS, Lee JW, Soung YH et al. Inactivating mutations of caspase-8 gene in colorectal carcinomas. *Gastroenterology* 2003; 125: 708-15.
33. Banelli B, Casciano I, Croce M et al. Expression and methylation of CASP8 in neuroblastoma: identification of a promoter region. *Nat Med* 2002; 8: 1333-5; author reply 5.
34. Pingoud-Meier C, Lang D, Janss AJ et al. Loss of caspase-8 protein expression correlates with unfavorable survival outcome in childhood medulloblastoma. *Clin Cancer Res* 2003; 9: 6401-9.