Identification of robust diagnostic and therapeutic target molecules for human malignancy is still an important issue. If we identify novel proteins which play a stem-line role for cellular transformation or aggravation of malignancy, it could give us a clue to diagnose a tumor in an earlier stage and to develop more reliable therapeutic tools. For this purpose, we have screened abnormally expressed genes in various human cancers by differential display RT-PCR. One of the overexpressed genes was a human cervical cancer oncogene (HCCR). HCCR was not only identified in cervical cancer tissues, but also found to be overexpressed in various human malignancies such as leukemia/lymphoma, breast, kidney, stomach, colon, liver and ovarian cancer. This molecule appeared to be a negative regulator of p53. In this paper, we discuss the biological functions of HCCR molecules and its implications for early diagnosis and future development of therapeutic devices of cancer.

Key words: DDRT-PCR, Oncogene, HCCR

Introduction

Cancer is a genetic disease caused by accumulation of multiple genetic abnormalities acquired congenitally or somatically and interaction with environmental factors (Balmain, 2002; Yarden et al., 2004). Neoplastic transformations, for example, are initiated by the aberrations of the genes for regulating cell proliferation, apoptosis, genome stability, angiogenesis, invasion or metastasis (Hanahan and Weinberg, 2000). Disruption of epigenetic regulation of single gene or genetic networks has also been known to be important for tumorigenesis (Jones, 2003; Hake et al., 2004).

Recent advances in the discovery of proto-oncogenes have contributed insights as to how cancer-associated genes are regulated and how we can deal with human malignancies. In 1983, researchers in Weinberg's laboratory converted normal human cells into tumor cells in a culture dish. They showed that the ectopic expression of the human telomerase catalytic subunit (hTERT) in combination with two oncogenes (the simian virus 40 large-T oncoprotein and an oncogenic allele of H-ras) resulted in direct tumorigenic conversion of normal human epithelial and fibroblast cells (Land et al., 1983). This achievement helped to identify new players in tumor formation and to understand complex processes of tumor development. In 1999, Weinberg's group made the first genetically defined human cancer cells (Hahn et al., 1999). Completion of human genome project and recent progress of research techniques such as global gene expression profiling by microarray, gene therapy techniques, and tumor vaccines have provided us with a better chance to understand and overcome this disease. However, for decades, basic and clinical research trials to cure cancers by blocking single cancer-associated molecule which has differentially expressed in the cancers have not been successful enough, because cancer is basically caused by multiple genetic abnormalities.

Nevertheless, some of the successful trials targeting single cancer-associated molecule have suggested the potential way for efficient treatment of malignancies. For example, pharmaceutical inhibitors of single oncoprotein such as tyrosine kinase inhibitor in BCR-ABL-positive chronic myeloid leukemia and farnesyltransferase inhibitors can inhibit the growth of some tumor cells such as mammary or salivary gland carcinomas (Deininger et al., 1997). Anti Bcl-2 oligonucleotide in non-Hodgkin lymphoma and c-myc antisense oligonucleotide in B-cell lymphoma also seemed to inhibit tumor cell growth (Chanan-Khan, 2004; Smith and Wickstrom, 1998). They indicate the importance of the reliable and sensitive identification of differentially expressed genes involved in the cellular transformation or tumor progression. If we could identify a gene playing pivotal roles for multiple essential steps of tumorigenesis, targeted inactivation of
that single gene could be sufficient for tumor regression. Concomitantly, it is also important to identify tumor antigens which stimulate a potent anti-tumor response. If this idea turns out to be true, a new era of immunotherapy against cancer will be heralded and eventually a powerful tumor vaccine can be developed. The novel oncogene can also be a target to develop a sensitive diagnostic tool for early detection of tumors. If serum antibody level against the cancer-associated molecule is significantly elevated or the novel protein is released into blood/urine/feces in cancer patients, they could be markers for early cancers. A reliable prognostic marker is another important point to predict clinical outcomes of cancers and to control tumors as well. Oncogenes or any overexpressed genes in a cancer might be a useful prognostic marker. Taken together, mining new cancer-associated genes is still a robust way to control human malignancies.

Through the population genetics studies, a lot of cancer-associated genes have been verified (Thompson et al., 2004). However, there will be even more unknown tumor-related genes, supposedly up to several hundred, yet to be found (Balmain, 2002). A number of approaches for the verification of differentially expressed genes have been developed. Among them, traditional northern blot analysis and RNase protection assay are a reliable approach, but global transcription profile analysis is not available with these tools. Other approaches such as serial analysis of gene expression, SAGE (Velculescu et al., 1995), gene expression microarray (Villeneuve and Parisenti, 2004), and differential display RT-PCR (DDRT-PCR) (Liang and Pardee, 1992) enabled us to do genome-wide global gene expression analysis.

We have studied the novel cancer-related genes which are expressed differentially in tumor tissue by DDRT-PCR approach. Recently we have identified several novel oncogenes including human cervical cancer oncogenes (HCCR) which were overexpressed in primary cervical cancers and cervical cancer cell lines (Ko et al., 2003) (Fig. 1a). HCCR oncogenes are classified into two types, HCCR1 and HCCR2, according to molecular characteristics (Fig. 2). These two proteins are alternative splicing variants. HCCR1 encodes 360 amino acids (~42 kDa) and HCCR2 encodes 304 amino acids (~36 kDa) molecules. As shown in figure 2, there are two potential N-myristylation sites (green dots), two potential phosphorylation sites for protein kinase C (pink dots), N-glycosylation site (blue dot), and hydrophobic trans-

**Differential display reverse transcriptase-PCR (DDRT-PCR)**

DDRT-PCR is a tool to compare gene expression levels between diseased and normal tissues (Liang et al., 1993). RNA from test and control tissue is reverse transcribed with degenerative oligonucleotide primers. Then the cDNA is PCR amplified with the random primers. During PCR, a radio-labeled nucleotide is incorporated into the amplicon. The PCR products are electrophoresed through the denaturing polyacrylamide gel and exposed to the film. Interpretation of differentially expressed gene is based on the comparison of band intensities between test and control samples. If the signal of the test sample is weaker than the control, the gene is interpreted as down-regulated, while if the test tissue band is stronger than the control, this gene is up-regulated during tumorigenesis. The up- or down-regulated bands are excised from the gel, eluted, re-amplified, and cloned in the vector. With the subsequent sequencing data, the cDNA is finally identified.

**Identification of HCCR**

We have screened the candidates of differentially expressed genes in primary cancer tissues, metastatic focus, and cancer cell lines by using DDRT-PCR (Kim et al., 2002; Ko et al., 2003; Ha et al., 2004). We have identified several novel oncogenes including human cervical cancer oncogenes (HCCR) which were overexpressed in primary cervical cancers and cervical cancer cell lines (Ko et al., 2003) (Fig. 1a). HCCR oncogenes are classified into two types, HCCR1 and HCCR2, according to molecular characteristics (Fig. 2). These two proteins are alternative splicing variants. HCCR1 encodes 360 amino acids (~42 kDa) and HCCR2 encodes 304 amino acids (~36 kDa) molecules. As shown in figure 2, there are two potential N-myristylation sites (green dots), two potential phosphorylation sites for protein kinase C (pink dots), N-glycosylation site (blue dot), and hydrophobic trans-

**Fig. 1.** Identification of novel oncogene HCCR by differential display RT-PCR. A. Differential display RT-PCR profiles of primary cervical cancer, metastatic focus of cervical cancer, and cervical cancer cell line CUMC-6 (Ko et al., 2003). HCCR was overexpressed in all cancer tissues and cell lines, with undetectable levels in normal cervical tissue. B. HCCR protein is overexpressed in various human malignancies. Upper box, comparison of HCCR expression level between normal tissue and various cancer tissues by northern blot analysis. Middle box, beta-actine expression level. Lower box, comparison of HCCR protein level between normal tissue and various cancer tissues by Western blot analysis.
Indeed, HCCR-overexpressed NIH/3T3 cells showed elevated transformation efficiency and more colony formation in soft agar (Ko et al., 2003). The HCCR transfected cells were found to be associated with p53 stabilization, decreased p53-responsive gene expressions such as p21 and Bax, and transdifferentiate the NIH3T3 cell to epithelial carcinomas. Figure 3 illustrates the potential functions of HCCR. To screen the HCCR interacting molecules, yeast two-hybrid analysis were performed. Several novel molecules have been identified and their functions are under evaluation.

Interestingly, HCCR1 and 2 are overexpressed in both RNA and protein level in various malignancies such as leukemia/lymphoma, breast, kidney, stomach, colon, and ovarian cancer (Fig. 1b,c). This implies that single oncogene HCCR may contribute to the development of multiple human malignancies. Although the multi-step malignant transformation process involving multiple genes/molecules has been a central dogma for tumorigenesis, HCCR overexpression in various malignancies and previously mentioned studies about anti-cancer effect by inhibiting single cancer-associated molecule (Deininger et al., 1997; Chanan-Khan, 2004; Smith and Wickstrom, 1998) suggests that there may be common stem-line changes in early step
tumorigenesis of various cancers. To verify this notion in in-vivo system, HCCR transgenic mice were generated (Ko et al., 2004). Mice transgenic for HCCR2 developed breast cancers including metastasis and p53 was negatively regulated in most organs. This evidence showed us that HCCR may play a stem-line role for the initiation of tumor development via inactivating p53 or further unidentified mechanisms. Therefore HCCR, as a stem-line molecule, can be an efficient target for the treatment or early detection of various human malignancies.

**Application of HCCR for early detection and treatment of malignancies**

Cancer is commonly associated with gain-of-function mutations affecting proto-oncogenes. Repairing these mutant genes or inactivating the oncogenic molecules may be useful in the treatment of cancer. However, since cancer is caused by multiple genetic abnormalities, inactivation of a single oncogene target may not be effective enough for tumor regression. It seems likely that the response of tumors to block a single pathway will vary according to the nature of the lesions, their contribution to tumorigenesis, the cellular lineage of the tumors, and the particular combination of further genetic lesions responsible for tumorigenesis.

Nevertheless, if HCCR plays a stem-line role for tumor development, there is a possibility to develop early/sensitive tumor detection devices and anti-tumor agents blocking the HCCR pathway. To validate whether HCCR molecule can be a sensitive diagnostic marker for cancers, HCCR1 polyclonal antibodies and monoclonal antibodies against HCCR$^{167-360}$ were generated and applied to human hepatocellular carcinomas (Yoon et al., 2004). HCCR protein was overexpressed in hepatocellular carcinomas compared with normal liver (Fig. 4). Liver cirrhosis also showed a weak positive signal. To examine whether the serum HCCR level can be a diagnostic marker for HCC, we made ELISA system to detect serum anti-HCCR antibody (Fig. 4d). The diagnostic accuracy of HCCR for HCC was significantly higher than that of alpha-fetoprotein. The sensitivity of HCCR was 78.2% which was significantly higher than that of alpha-fetoprotein and the specificity of HCCR for the diagnosis of HCC was 95.7%. Altogether, serum HCCR level was proven to be highly sensitive and specific for the diagnosis of HCC. Therapeutic applications of HCCR for various types of cancers are under review.

**Perspectives**

Although several putative biological roles of HCCR have been partly revealed, there must be more biological functions to be discovered. For example, the mechanism of HCCR activation has not been proven yet. If we uncover the mechanism, it will give us a clue to prevent or treat HCCR mediated malignancies. Screening HCCR interacting molecules is one of the essential ways to understand the biological function of HCCR. With the results, we can simultaneously try gene therapy (antisense and peptide nucleic acid therapies), anticancer drug development (RNA, combinatorial and chemical libraries), and cancer vaccine. Figure 5 illustrates the future diagnostic and therapeutic application of HCCR.
References


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