Symposium on Present Status of Clinical Oncology*

Moderator: Kaoru ABE

National Cancer Center, Tokyo

Moderator: Ryuzo UEDA

The Second Department of Internal Medicine, Nagoya City University School of Medicine, Nagoya

1. Clinical Application of Molecular Diagnosis for Cancer Patients

Ryuzo UEDA

The Second Department of Internal Medicine, Nagoya City University Medical School, Nagoya

Key words: molecular diagnosis, cancer, minimal residual disease (MRD), chromosomal translocation, polymerase chain reaction (PCR)

Great advances in molecular biology have contributed to the understanding of the mechanisms of the oncogenesis and clonal nature of neoplasia with tumors arising from single transformed cells by accumulation of genetic alterations in multi-steps (Table 1) (1).

Traditionally, the diagnosis of cancer was made by pathologists based on morphology and other phenotypic criteria including differentiation antigens and tumor markers. However, recent molecular technology has provided us with useful in-

formation about specific genetic alterations in tumors. Thus, molecular diagnosis can be a powerful adjunct to histopathology, helping 1) detect malignant cells with clonal markers, 2) understand the heterogeneity of individual tumors, 3) monitor the response to cancer therapy and evaluate prognosis.

The molecular approaches utilized in cancer diagnosis and their sensitivities are summarized in Table 2. There are three characteristic features of molecular diagnosis. First, the sensitivity is high, so that small sample is sufficient. Second, the result is exact and reproducible and therefore objective, and third, results can be obtained very rapidly. The advantages in sensitivity, objectivity and rapidity are of essential significance for clinical diagnosis.

Detection of a malignant cell

The first application is in the primary diagnosis of cancer, to determine whether samples obtained from biopsied specimens, blood, sputum, urine etc., contain a malignant cell or not. Determination is based on the finding of clonal cells within samples examined. In lymphoid malignancies, the detection of clonal rearrangement bands of Ig genes including complementarity determining regions (CDR) and T cell recep-

Table 1. Molecular Oncogenesis

I. Genetic alteration
(1) chromosomal translocation
(2) deletion, insertion
(3) point mutation
(4) amplification
II. Epigenetic alteration
(1) DNA methylation and demethylation
(2) RNA splicing
(3) telomerase

*Presented at the 95th Annual Scientific Meeting of the Japanese Society of Internal Medicine, April 10, 1998, Fukuoka

Reprint requests should be addressed to Dr. Ryuzo Ueda, the Second Department of Internal Medicine, Nagoya City University Medical School, Kawasumi, Mizuho-ku, Nagoya 467-8601

Table 2. Methods for Detection of MRD in Hematologic Malignancies

	Relative Sensitivity	Advantages	Disadvantages
I. Cytogenetics	$10^{-1}(?)$	Specific marker	Insensitive Require proliferation
II. Southern analysis	10^{-2}	Rapid	Insensitive Limited application
III. FISH	10^{-2} – 10^{-3}	Rapid Specific marker	Relatively insensitive High background
IV. FACS	10^{-2} – 10^{-4}	Rapid Relatively sensitive Quantitative	Phenotype switch Require viable cells
V. PCR	10^{-4} – 10^{-6}	Rapid Highly sensitive Highly quantitative	Potential contamination Low mRNA expression

tor genes, has been applied in clinical use. It is impossible to diagnose the benign/malignant boundary diseases such as angioimmunoblastic lymphadenopathy with dysproteinemia (AILD), T-zone lymphoma, and reactive lymphoreticular hyperplasia (RLH), without data on monoclonality (2). Detection of monoclonal integration of human T-cell leukemia virus type-1 (HTLV-1) proviral DNA is essential for the diagnosis of adult T-cell leukemia (ATL) in some cases. The human androgen receptor (HUMARA) gene is sometimes very useful to determine the clonality of solid tumors in females. p53 is mutated most commonly in cancer tissue (3). If two lesions show different mutations among the same organs, such as lung, it is likely that these lesions are derived from independent clones, thus, they are not metastases. These kinds of information are very useful in order to design a treatment strategy appropriate for the individual patient (4).

Heterogeneity within individual tumors

The heterogeneity of tumor cells is one of the most characteristic features of neoplasms. An understanding of the genetic changes in tumor cells has cast light on the mechanisms responsible for biological heterogeneity. This is closely related to the clinical grade of malignancy such as stage, the capacity of progression and the sensitivity to chemotherapy. With hematopoietic malignancies, more than 70% have been shown to possess chromosomal translocations which are closely associated with certain types of leukemia or lymphoma (5, 6). There are two possible consequences of translocations, one being generation of chimeric gene products which produce chimeric proteins. The other is deregulation of genes encoding normal transcripts translated into normal proteins. There are many representative examples. For the former, BCR/ABL in chronic myeloid leukemia, PML/RAR α in acute promyelocytic leu-

Figure 1. Molecular mechanisms of chromosomal translocation.

kemia and MLL/LTG4, 9, 19 in infant and therapy-related leukemia can be cited. For the latter Myc translocation in Burkitt's lymphoma, BCL-1 translocation in mantle cell lymphoma, and BCL-2 in follicular lymphoma are good examples (Fig. 1). It is generally accepted that the treatment decision must be made according to the genotype of the disease in hematological malignancies.

Some oncogenes in solid tumors, for example the N-myc gene in the diagnosis of neuroblastoma, erbB2/neu/HER-2 in breast and ovarian carcinomas have been utilized for evaluation of the prognosis. So far, currently available molecular markers for clinical use are limited, but many studies have sug-

gested that an increasing number of somatic alterations leads to biological heterogeneity of tumors and effect on clinical behavior and stage.

Monitoring of minimal residual disease (MRD) to evaluate the response to cancer therapy

MRD can now be analyzed using either polymerase chain reaction (PCR)-based assays or FISH analysis. An advantage using the PCR is in its high sensitivity of 10^{-4} to 10^{-6} with respect to detecting target sequences. Monitoring MRD is exclusively powerful for hematopoietic malignancies, since chromosomal translocations, which can be specific tumor mark-

Figure 2. Monitoring MRD by detecting BCR/ABL in Ph ALL patients.

Figure 3. Cancer early detection and MRD.

ers, play major roles in the development of leukemia/lymphoma, as mentioned above.

In chronic myeloid leukemia (CML) and a fraction of acute lymphoid leukemia (ALL) cases, BCR-ABL fusion transcripts resulting from the Philadelphia chromosome (Ph), can be a hallmark of residual leukemic cells. As for acute promyelocytic leukemia (APL) bearing a PML-RAR α fusion gene and the therapy-related leukemia bearing mixed lineage leukemia (MLL)-translocation related fusion genes (TRG), the clinical significance of MRD after treatment is now being assessed as a project supported by the Ministry of Health and Welfare in Japan. Our data indicate that MRD exceeding a critical level usually precedes clinical relapse and, in addition, complete elimination of leukemic cells is not always necessary for cure (Fig 2)(7).

Recently the WT1 gene as a molecular marker for MRD detection in leukemia patients with the PCR procedure has been demonstrated. This assay has a possibility to become widespread to evaluate the MRD irrespective of the type of leukemia because aberrant expression of the WT1 gene is not associated with specific oncogenes (8).

In summary, research in molecular biology has provided us with new insights into diagnosis of various malignancies and monitoring MRD after treatment. In the future, it is crucial to develop accurate methodology to determine the amounts of residual tumor cells, by which clinical outcomes including onset of disease and relapse can predict and eventually to develop

the complete cure-oriented therapy for malignancies (Fig 3). Thus, it will be necessary for physicians to transfer basic understanding of genetic changes in malignancies from the bench to the bedside, especially with due consideration to ethical and social problems (9).

References

- 1) Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. *Science* **278**: 1043–1050, 1997.
- 2) Takagi N, Nakamura S, Ueda R. et al. A phenotypic and genotypic study of three node-based, low-grade peripheral T-cell lymphomas: angioimmunoblastic lymphoma, T-zone lymphoma, and lymphoepithelioid lymphoma. *Cancer* **69**: 2571–2582, 1992.
- 3) Lane DP. Cancer. p53, guardian of the genome. *Nature* **358**: 15–16, 1992 (news; comment) (see comments).
- 4) Sidransky D. Advances in cancer detection. *Sci Am* **275**: 104–109, 1996.
- 5) Rabbitts TH. Chromosomal translocations in human cancer. *Nature* **372**: 143–149, 1994.
- 6) Look AT. Oncogenic transcription factors in the human acute leukemias. *Science* **278**: 1059–1064, 1997.
- 7) Ni H, Nitta M, Komatsu H. et al. Detection of bcr/abl fusion transcripts by semiquantitative multiplex RT-PCR combined with a colorimetric assay in Ph positive leukemia. *Cancer Lett* **124**: 173–180, 1998.
- 8) Inoue K, Ogawa H, Yamagami T. et al. Long-term follow-up of minimal residual disease in leukemia patients by monitoring WT1 (Wilms tumor gene) expression levels. *Blood* **88**: 2267–2278, 1996.
- 9) Solter D. Dolly is a clone-and no longer alone. *Nature* **394**: 315–316, 1998 (news; comment) (see comments).

2. New Molecular Targets for Cancer Chemotherapy

Shuji NAKANO

The First Department of Internal Medicine, Kyushu University, Fukuoka

Key words: molecular target, cancer chemotherapy, molecular biology, chemotherapy-refractory cancer

Introduction

The principle of cancer chemotherapy is aimed toward total kill of the cancer cells. The discovery of new anti-cancer drugs capable of eradicating cancer cells more efficiently than normal cells has contributed improvement of cancer chemotherapy, resulting in the cure of chemosensitive cancers. For this purpose, maximum tolerated doses of anticancer drugs are administered. However, the patients who can obtain benefits from such high-dose chemotherapy is limited to a small population of cancers. Cancer chemotherapy is usually not effective in frequently encountered human malignancies in Japan, such as

gastric, lung and colorectal cancers. For the breakthrough of these problems inherent in conventional cancer chemotherapy, the concept of molecular target therapy for cancers has emerged, which involves treatments of cancers with inhibitors or modulators of the molecules which specify cancer cell properties. This is a theoretical treatment option which is quite different from conventional approaches, aimed not toward total cell kill, but to promoting survival of the patients with cancer. In this review, the significance and strategy of the recent advances in molecular target therapy are presented and discussed.

Reprint requests should be addressed to Dr. Shuji Nakano, the First Department of Internal Medicine, Kyushu University, Maidashi, Fukuoka, Higashi-ku, Fukuoka 812-0053