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Cancer Precision Medicine: Physiological Function of C-MYC as Targeted Molecule

Takuma Hayashi[1,4], Ikuo Konishi[1,2,3]

[1]National Hospital Organization Kyoto Medical Center, Kyoto, Kyoto, Japan
[2]Kyoto University School of Medicine, Kyoto, Japan
[3]Immediate Past President of Asian Society of Gynecologic Oncology, Japan
[4]D.M.Sci., M.S., Section Head, Cancer Medicine, National Hospital Organization, Kyoto Medical Center, Fushimi-Ku, Kyoto City, Kyoto, Japan

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ABSTRACT

The genome represents a design for creating the body, with each one being different. In cancer genomic medicine, many genes are simultaneously examined using mainly cancer tissues (the oncogene panel test), and gene mutations are revealed. Cancer treatments are then initiated according to each individual's constitution and medical condition based on gene mutations. A system for cancer genome medical treatment is currently being developed. In the treatment of several cancer types, the "oncogene test with an oncogene companion diagnosis" is already being performed as a standard test using cancer tissue to detect one or several gene mutations. Precision Medicine: discovering unique therapies that treat an individual's cancer based on the specific abnormalities, i.e. germline or somatic mutations of their tumors. In this paper, we will explain the biological role of C-MYC and emphasize the importance of C-MYC as a target factor in cancer precision medicine. The functional activated C-MYC for cell proliferation and tumorigenesis is potential candidate as anti-oncogenic molecule.

KEYWORDS: *C-MYC, gene expression, precision medicine, hepatocyte, partial hepatectomy*

Precision medicine is an approach to patient care that allows doctors to select treatments that are most likely to help patients based on a genetic understanding of their disease. This may also be called personalized medicine.

The idea of precision medicine is not new, but recent advances in science and technology have helped speed up the pace of this area of research. Today, when patients are diagnosed with cancer, patients usually receive the same treatment as others who have same type and stage of cancer. Even so, different patient may respond differently, and, until recently, doctors didn't know why. After decades of research, scientists now understand that patients' tumors have genetic changes that cause cancer to grow and spread. They have also learned that the changes that occur in one person's cancer may not occur in others who have the same type of cancer. And, the same cancer-causing changes may be found in different types of cancer.

Myelocytomatosis oncogene (MYC) was initially discovered in the form of a viral oncogene of an avian myelocytomatosis virus, MYC 29, and subsequently identified in various vertebrate genomes in the forms of its cellular counterpart, C-MYC, and transducing viral MYC oncogene homologue (v-MYC) in several oncogenic retroviruses [1,2]. The C-MYC protooncogene encodes a DNA-binding factor that can activate and repress transcription. Via this mechanism, C-MYC regulates expression of numerous target genes that control key cellular functions, including cell growth and cell cycle progression. C-MYC also has a critical role in DNA replication. Deregulated C-MYC expression resulting from various types of genetic alterations leads to constitutive C-MYC activity in a variety of cancers and promotes oncogenesis [3]. Research experiment showed that the normal human homolog of the avian *MYC* oncogene was present in multiple copies in the DNA of a malignant promyelocyte cell line derived from the peripheral blood of a patient with acute promyelocytic leukemia [4]. Other human oncogenes were not amplified.

Contrary to the previous belief that C-MYC is wildtype in both types of tumors, 65% of 57 Burkitt lymphomas and 30% of 10 mouse plasmacytomas reportedly exhibited at least 1 amino acid substitution [5]. These mutations were apparently homozygous in all Burkitt lymphoma biopsies, implying that the mutations often occur before C-MYC/Ig (OMIM 147220) translocation. In the mouse plasmacytomas, only the mutant *MYC* allele was expressed, indicating a functional homozygosity with occurrence of mutations at the translocation. Many mutations were clustered in regions associated with transcriptional activation and apoptosis, and in the Burkitt lymphomas, they frequently occurred at sites of phosphorylation, suggesting that the mutations had a pathogenetic role. Most of the mutations were clearly not polymorphisms, for reasons in addition to the large number

of different mutations observed: 1) a high proportion were missense mutations; 2) most tumors contained multiple mutations; and 3) each tumor had a unique pattern of mutations.

We examined differential expression of *c-Myc* mRNA in hepatocytes after partial hepatectomy in order to understand molecular process of *c-Myc* gene expression. Our findings suggest the existence of a short-lived protein, which suppresses the expression of *c-Myc*. In an attempt to identify these putative regulatory elements, we mapped DNase I hypersensitive sites (HSs) in the rat *c-Myc* locus in hepatocyte**s** after partial hepatectomy. In functional *in vivo* analyses, we elucidated the chromatin structure and potentiality of regulating factor(s) for *c-Myc* gene expression.

Rats were killed at various times after partial hepatectomy and total RNA was extracted from their regenerating liver [6,7]. Figure 1 shows the results of analysis of the RNA with the 3'-half fragment of *v-Myc* as a probe [8,9]. Essentially similar results were obtained when a cloned fragment containing exon 1 of the rat *c-Myc* gene was used as a probe. As shown in Figure 1, the amount of the 2.5-kilobase transcript of *c-Myc* had already increased 30 min after the operation; it reached a maximum at 1-3 hours and then decreased. The bands were traced with ImageJ and quantified from their peak areas. Figure 2 compares these values with those of normal liver, together with the time course of incorporation of ^{3}H-thymidine into the acid-insoluble fraction of the liver, when injected intraperitoneally 1 hour before death. At 30 minutes, the amount of *c-Myc* transcript was 10-fold that in control liver, increased to a maximum of 10-15-fold at 1 hour and decreased after 4 hours. The decrease was rapid and *c-Myc* transcripts had returned to approximately normal levels after 8 hours, although DNA synthesis had not begun to increase.

The expression of *c-Myc* gene was reportedly increased three to five-fold at 12-18 hours after partial hepatectomy. In our results over the same period, levels of *c-Myc* gene expression were also high, although they were never more than double that in normal liver. Examination of *c-Myc* gene expression at earlier stages of the regeneration revealed a conspicuous peak soon after partial hepatectomy, whereas the previous report did not determine the expression of *c-Myc* gene at that time. We also examined the expression of the *Harvey ras* (*H-ras*) gene during liver regeneration using the same filters as for analysis of the expression of *c-Myc* gene. In accordance with earlier reports, we observed an increase in the expression of *H-ras* gene, but this became evident after 8 hours, and peaked at a level two to three times that in normal liver, at about 30 hours after partial hepatectomy [10-12]. *In vitro* stimulation of B lymphocytes, T lymphocytes or cultured fibroblasts with their respective specific mitogens is known to induce an immediate increase in the expression of *c-Myc* gene [13]. This increase is temporary, and the expression returns to the uninduced level by the time DNA replication starts. C-MYC induction is not blocked by inhibition of protein synthesis but is instead enhanced in mitogen-stimulated cells *in vitro*.

The present findings show that the same is true for an in vivo system in which differentiated resting cells are stimulated to proliferate. We examined the effect of inhibition of protein synthesis on the expression of *c-Myc* gene in regenerating liver. We observed a 100-fold increase in *c-Myc* transcription in samples from rats treated with cycloheximide 1 hour before partial hepatectomy and killed 2 hours after the operation (CH). The amount of *H-ras* transcript was not significantly changed by cycloheximide treatment. Therefore, the increase in the amount of *c-Myc* transcript observed in cycloheximide treated liver is likely to be the result of enhanced synthesis rather than of stabilization of mRNA in general [9,14]. However, other possibilities such as specific stabilization of *c-Myc* mRNA cannot be excluded. Interestingly, a similar increase has also been found in a sample from a rat without partial hepatectomy treated 3 hours previously with cycloheximide. This effect of cycloheximide alone was prolonged and was even increased by about 600-fold at 6 hours after treatment with and without partial hepatectomy. Thus, the mode of induction of C-MYC by cycloheximide seems to be different from that in regenerating liver, where the induction is temporary and the extent of induction is increased up to 10-15-fold. Enhanced induction by cycloheximide was also observed when treatment was preceded by partial hepatectomy. Hence, inhibition of protein synthesis seems to block the switch-off of *c-Myc* transcription observed at 4 hours or later after partial hepatectomy.

To identify additional regulatory elements in the *c-Myc* locus, we performed DNase I hypersensitive site (HS) analyses of hepatocytes after partial hepatectomy. Examination of the C-MYC chromatin in hepatocytes after partial hepatectomy would allow detecting potential tissue specific difference in DNase I hypersensitivity. DNA from DNase I treated nuclei of hepatocyte**s** after partial hepatectomy was initially digested with *Sac I* and evaluated for location of HSs by Southern blot hybridization using probe pGEMmyc1 (Fig. 3). Similar studies of the *c-Myc* chromatin have so far been confined to the coding and immediate downstream region of the gene, which was digested with *Hind III* (Fig. 3). From the broad panel of different examined cell lines, we conclude that transcriptionally active *c-Myc* genes exhibit this pattern of DNase I HSs (Fig. 3). As shown in Figure 3, cleavage by DNase I created additional, smaller subfragments, corresponding to previously unidentified hypersensitive sites located within first exon and first intron. Following the nomenclature of the HSs in the promoter region and first intron of *c-Myc* gene, we designated the three most prominent HS sites I, II, and III in upstream region of *c-Myc* first exon and I*, II*, and III* in *c-Myc* first intron (Fig. 3).

As shown here and in an earlier report, *c-Myc* transcription is increased immediately after the cells have been stimulated to proliferate, but this expression of *c-Myc* stops soon after it has reached a maximum. Because inhibition of protein synthesis enhances the induction of C-MYC, it seems likely that *c-Myc* gene is repressed by a short-lived protein that becomes abundant soon after the onset of the proliferative process. Based on observations in Burkitt's lymphoma cells in which *c-Myc* genes were translocated to immunoglobulin gene loci, Leder *et al*. have elaborated several possible models concerning the regulatory mechanisms of *c-Myc* gene [15]. Among these, the auto regulatory model is the simplest, yet it is consistent with the facts that *c-Myc* gene is expressed transiently following inductive stimulation and that *c-Myc* mRNA is induced by the inhibition of protein synthesis. In *Eschericha coli*, analogous auto regulatory mechanisms have

been described for a stress protein, dnaK, and for a repressor protein in the SOS function, lexA, which becomes extensively but transiently expressed after inductive stimuli [16,17]. The induction following the proliferative signal can be explained by supposing that the signal activates a process, such as modification of C-MYC protein, thereby abolishing its repressor activity. Expression of C-MYC at a low but distinct level in various tissues is also consistent with auto regulation [18]. Another possibility is that expression of *c-Myc* gene is repressed by some other "early gene" products [19]. Such gene products are expressed transiently at an early stage in mitogen-stimulated fibroblasts and the levels of their mRNA are enhanced by inhibition of protein synthesis.

We have found previously that in all chemically induced primary hepatomas examined, the level of *c-Myc* transcript was three to five times that in normal liver or normal liver tissue adjacent to the tumor [12]. Altered regulation of *c-Myc* gene in some B-cell lymphomas and in other tumor cells as a result of translocation, viral enhancer insertion or gene amplification are well established phenomena [20,21]. Recently, in normal fibroblasts, C-MYC was reportedly induced by growth stimulation, but that it was constitutive in two chemically transformed derivatives of fibroblasts [22,23]. These facts strongly suggest that altered regulation, and perhaps abnormal increase in the expression of C-MYC, might prevent the cells from entering the G0 phase and thus lea to their infinite growth. Further studies on the regulation of mutant C-MYC as well as normal C-MYC increase our understanding of the processes involved in the development of cancer (Fig. 4).

C-MYC, oncogene as well as its paralogs *MYCN* and *MYCL1*, has been shown to play essential roles in cycling progenitor cells born from proliferating zones during embryonic development. After birth, MYC plays important roles in the proliferation of all cell types. *MYC, MYCN*, and *MYCL1* amplifications have all been described in malignancy associated with poor prognosis (Fig. 4). C-MYC represents one of the most sought-after drug targets in cancer. C-MYC transcription factor is an essential regulator of cell growth in most cancers. Over 40 years of research and drug development efforts did not yield a clinically useful C-MYC inhibitor [24,25]. Chronological development of small-molecule MYC prototype inhibitors and corresponding binding sites are comprehensively reviewed and emphasis is placed on modern computational drug design methods. On the outlook, technological advancements may soon provide the so long-awaited MYC clinical candidate for precision medicine in cancer therapy.

Conflicts of interest:
We do not have any conflicts of interest.

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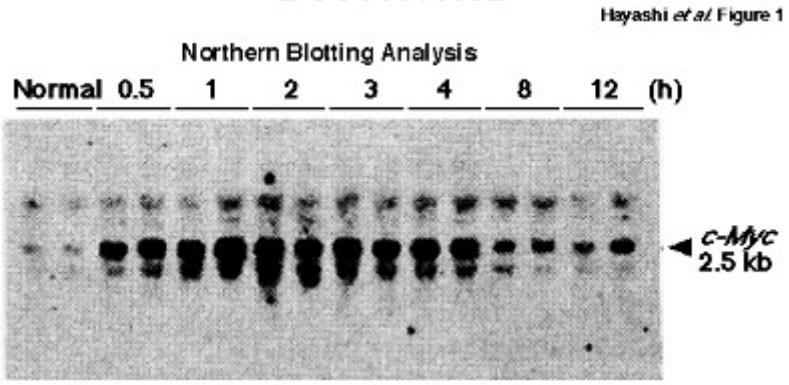

Figure 1 Levels of rat *c-Myc* transcripts in the liver at various times after partial hepatectomy.

Male Sprague-Dawley (SD) Rats (200-250 g, CHARLES RIVER LABORATORIES JAPAN, INC., Kanagawa, Japan) were partially hepatectomized under ether anaesthesia following the method [2]. The rats were killed at the indicated times after the operation and total RNA was extracted from the liver by guanidium thiocyanate hot phenol method [18]. The RNAs (10 μg per one sample) were separated on 1.2% agarose gel containing 6% HCHO3, blotted onto a nitrocellulose membrane filter and hybridized to a nick-translated 3'-half *SalI-Pst1* fragment of *v-Myc*, which was [α-^{32}P]dATP-labelled [4]. Each sample represents an RNA sample from one rat. After exposure, the probe was removed and the RNA was re-hybridized to [α-^{32}P]dATP-labelled *v-Ras* and mouse rDNA sequences. These probes were insert fragments of plasmids BS9 (ref. 25) and p6.6 (ref. 26), respectively. The size of rat *C-Myc* trtanscript was estimated from the position of its bands relative to that of rRNA.

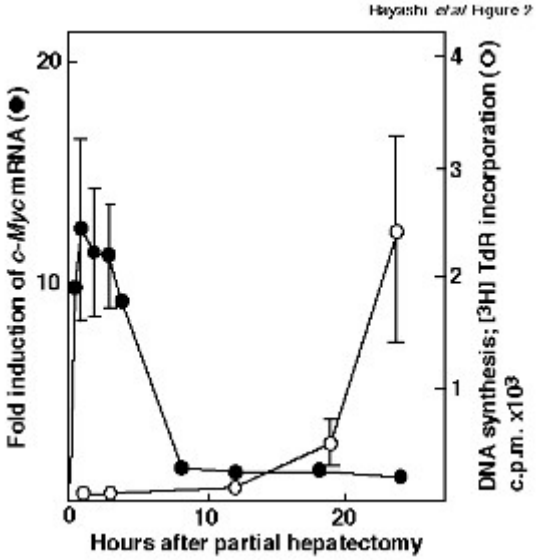

Figure 2 Relationship between the appearance of *C-Myc* transcript and DNA synthesis in regenerating rat liver.

The Fuji imaging file shown in Fig.1 and the autoradiogram of rRNA of the same filter were scanned with a ImageJ. The amount of *c-Myc* transcript in each sample relative to that of 28S rRNA of the same lane was determined and expressed relative to the value for control liver. The RNAs to DNAs ratio did not change appreciably during the 19 hours of rat liver regeneration. Because rRNA represents the majority of the total RNA, the relative amount of *c-Myc* mRNA per DNA and therefore per cell in each samples, can be approximated by the relative ratio of c-myc transcript to 28S rRNA. DNA synthesis was measured as follows. Rats were injected intraperitoneally with ^{3}H-thymidine (TdR) at 100 μCi per rat 1 hour before death. Their livers were homogenized in 20-fold excess of the PK buffer described by Favaloro *et al.* [21]. One hundred μL of the homogenates were spotted onto a glass fiber filter and their acid-insoluble radio activities were counted. The points shown are the average of duplicates with the ranges indicated.

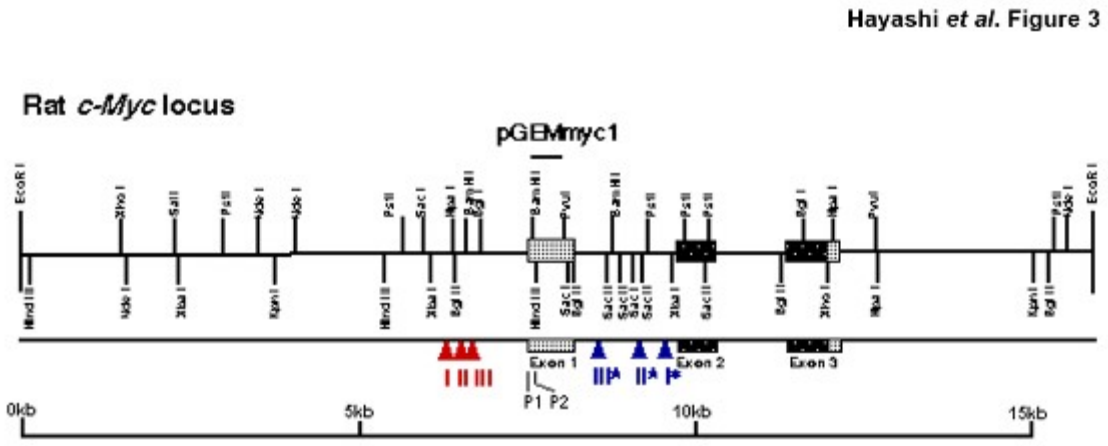

Figure 3 Mapping of HSs of rat *c-Myc* locus.

DNase I treated and *Sac I* or *Hind III* digested DNA samples were probed with pGEMmyc1. As shown here for hepatocytes from rats, which were killed 2 hours after partial hepatectomy, HSs located in upstream from *C-Myc* first exon and in *C-Myc* first intron were marked with roman numeral; I, II, III, I*, II*, and III*.

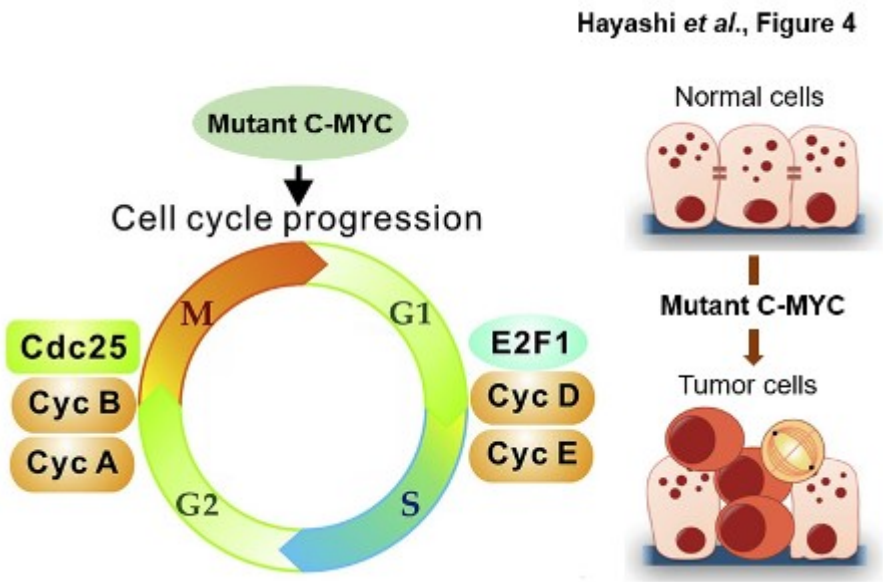

Figure 4 Significance of C-MYC in cell cycle.

Soon after the discovery of the *MYC* gene (C-MYC), it became clear that MYC expression levels tightly correlate to cell proliferation. The entry in cell cycle of quiescent cells upon MYC enforced expression has been described in many models. Also, the down regulation or inactivation of MYC results in the impairment of cell cycle progression. Given the frequent deregulation of MYC oncogene in human cancer it is important to dissect out the mechanisms underlying the role of MYC on cell cycle control. Activated mutant C-MYC dramatically induces epithelial transformation.