PREFACE (REVISION)

The revised retirement commemorative monograph, the first edition of which was uploaded in 2015, describes our important and remarkable findings as follows. When higher eukaryotic cells encounter an abnormal and uncomfortable intra- and/or extra-cellular environment change in their lives, in order to adapt for and/or eliminate it, they possess an ability to gain un-programmed and new cell functions by means of irreversible creation of chromatin structure plasticity with epigenetic modifications through various generations. Outline of our results is concretely as follows.

In the chicken DT40 cell line, histone deacetylase-2 (HDAC2) indirectly regulates gene expressions of IgM H- and L-chains through opposite regulations of gene expressions of Pax5, Aiolos, EBF1, OBF1, and Ikaros plus E2A. The HDAC2-deficiency in DT40 cells induces dramatic accumulations of mRNAs and proteins of IgM H- and L-chains. Thereafter these accumulated immunoglobulin mRNAs and proteins are dramatically reduced in almost similar changing pattern in all individual clones of HDAC2(-/-) DT40 mutants during continuous cultivation. By contrast, gene expressions of Pax5, Aiolos, EBF1 and OBF1 remarkably show distinct changing patterns in individual clones of HDAC2(-/-) DT40 mutants during continuous cultivation. At the later stage of cultivation, there exist at least three distinct ways for gene expressions of IgM H- and L-chains, i.e., OBF1-dependent, Pax5- and Aiolos-dependent, and Pax5-, Aiolos- and EBF1-dependent types. These distinct alterations in gene expressions of Pax5, Aiolos, EBF1 and OBF1 in individual clones of HDAC2(-/-) DT40 mutants are originated from the varied chromatin structure plasticity surrounding their proximal 5'-upstream regions. The chromatin structure plasticity is irreversibly created through successive chromatin conformation (structure) changes based on varied changes in acetylation levels of specific Lys residues of histone H3 in individual mutant clones during cultivation. Based on these results, we clarified ways to diminish accumulated IgM H- and L-chains by means of irreversible creation of the varied chromatin structure plasticity surrounding the proximal 5'-upstream regions of the above-mentioned specific transcription factor genes with acetylation and deacetylation of specific Lys residues of histone H3 in individual clones of HDAC2(-/-) DT40 mutants during cultivation.

Furthermore, we proposed a universal hypothetic way, which we named the chromatin conformation change code (4C) theory, for the bio-system to gain un-programmed and new cell functions by means of irreversible creation of the chromatin structure plasticity with epigenetic modifications through various generations in higher eukaryotes. Outline of the 4C theory is concretely as follows. Somatic cells of higher eukaryotes are pluri-potent, elastic and flexible for gaining un-programmed and new cell functions by means of irreversible creation of the chromatin structure plasticity, in order to adapt themselves to an abnormal intra- and/or extra-cellular environment change. The pluri-potency, elasticity and flexibility of somatic cells for an ability to gain un-programmed and new cell functions are fundamentally originated from those of the chromatin structure. The chromatin structure plasticity (the loose or tight form)

surrounding the proximal 5'-upstream regions of specific transcription factor (and chromatin-modifying enzyme) genes are created by means of their irreversible conformation changes with epigenetic modifications through various generations (cell divisions). The chromatin structure of proximal 5'-upstream region, as just dynamic and changeable three-dimensional conformation, possesses two fundamental abilities, i.e., to receive signal concerning an abnormal environment change and to direct the switch (on or off) for latent transcription ability of the corresponding gene. Variety in irreversible creation of the chromatin structure plasticity among individual cells is triggered by the initial spontaneous unbalanced response to the abnormal environment change and accomplished by the successive convergence of the response throughout various cell divisions. Thus, individual somatic cells of higher eukaryotes can gain the same or different un-programmed and new cell functions in distinct ways depending on the varied chromatin structure plasticity, even though they are the same cell type.

The 4C theory, which opens the door for gaining un-programmed and new cell functions of higher eukaryotes and innovates the general concept concerning somatic cells, is the typical fruit of my small serendipity and is also the outcome of some 10 years of a small research group (of T. N. and M. N.). We had undertaken the research on the 4C theory, which made my heart beat fast with joy, under the worst research conditions, i.e., without any grants, in aperture of our other projects and only by a womanpower (of M. N.). While I was in active service, to my regret, all of data on the 4C theory could not been published as papers in journals and presentations in meetings. Moreover, I could not obtain additional data on the 4C theory any more, due to my retirement. Therefore, on the occasion of my thorough retirement, I seriously started writing of rough drafts and manuscripts of our original monograph on the 4C theory as the form of original papers in series. Anyhow, I have a tiny credit for the 4C theory, which is a creative hypothetic concept on an ability of higher living things for gaining un-programmed and new cell functions, because all of these studies on the theory were originally started and achieved only by my small group. Finally, I earnestly crave that somebody is interested in and takes over the research on the 4C theory in the near future.

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CONTENTS

ABBREVIATIONS

Amp: ampicillin

ASF1: anti-silencing function 1

Blimp1: B lymphocyte-induced maturation protein-1

BSA: bovine serum albumin

CAF-1: chromatin assembly factor-1

4C machinery: chromatin conformation change complex machinery

4C theory: chromatin conformation change code theory

ChIP: chromatin immuno-precipitation

DAB: 3', 3'-diaminobenzidine

E2A: E box binding protein 2A

EBF1: early B cell factor 1

ECRR/ECRS: environment change recognition receptor/site

EDTA: ethylenediaminetetraacetic acid

F, E, M or L: first, early, middle or later (stage of cultivation)

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

GCN5: general control non-depressible 5

HIRA: a homolog of S. cerevisiae transcriptional corepressors Hir1p and Hir2p

HAT: histone acetyltransferase

HDAC: histone deacetylase

HRP: horseradish peroxidase

Hyg: hygromycin

IgM Hc, Hm or Hs: whole, membrane-bound or secreted form of IgM H-chain

K9/H3, K14/H3, K18/H3, K23/H3 or K27/H3: Lys-9, Lys-14, Lys-18, Lys-23 or Lys-27 residue of histone H3

NotchIP: neighboring overlapping tiling chromatin immuno-precipitation

NHS: normal horse serum

OBF-1: origin binding factor-1

Pax5: paired box gene 5

PB: phosphate buffer

PBS: phosphate buffered saline

PCAF: p300/CBP-associated factor

PCR: polymerase chain reaction

PMSF: phenylmethylsulfonyl fluoride

PU.1: purine box factor 1

RT-PCR: reverse transcription-polymerase chain reaction

SDS: sodium dodecyl sulfate

SDS-PAGE: SDS-polyacrylamide gel electrophoresis

TB: Tris-HCl buffer (pH 7.4)

TCA: trichloroacetic acid

TE: 10 mM Tris-HCl, 1 mM EDTA (pH 8.0)

Tet: tetracycline

TFC machinery: transcription factor complex machinery

2D-PAGE: two-dimensional polyacrylamide gel electrophoresis

W: wild-type (DT40 cells)

XBP-1: X-box binding protein-1