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分担研究報告書

酸化ストレスに起因する発がんの抑制に関する分子遺伝学的研究

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研究要旨
Msh2 遺伝子欠損マウスを用いて、臭素酸カリウム（$KBrO_3$）の飲水投与による消化管での酸化ストレス誘発発がん実験を行い、酸化ストレスを付与された *Msh2* 遺伝子欠損マウスの腸管では、顕著に上皮性腫瘍の発生頻度の上昇が認められた。これらの結果から、MSH2 は酸化ストレスに起因する発がんの抑制に重要な役割を果たしていることか明らかになった。通常の環境で飼養した野生型マウスの突然変異解析の結果、腸管上皮細胞では酸化ストレスに起因する G:C→T:A 変異が加齢とともに顕著に増加する。今回の結果と合わせて考えると、ミスマッチ修復系遺伝子の欠損によって引き起されるヒト遺伝性非腺腫性大腸がん（HNPCC）発症には、腸管で生じる酸化ストレスが要因であることが強く示唆される。

Ａ．研究目的

これまでの研究で、私達は遺伝子欠損マウスを用いて、*MUTYH* 遺伝子に欠損を持つ MAP 患者では酸化ストレスに起因する自然突然変異が大腸腺腫症を引き起こすことを示した。同時に、臭素酸カリウム（$KBrO_3$）の飲水投与による、消化管での酸化ストレス誘発発がん系を確立した。本研究では、この系を用いて、ミスマッチ修復系が酸化ストレスに起因する発がんの抑制に果たす役割を解明し、ヒト遺伝性非腺腫性大腸がん（HNPCC）の発がん機序を考察する。

Ｂ．研究方法

1） *Msh2* 遺伝子欠損マウスの飼養
近交系 C57BL6/J の遺伝的背景を持つ *Msh2* 遺伝子欠損マウスのヘテロ接合体同士の掛け合わせにより *Msh2* 遺伝子欠損マウスと対照群の野生型マウスを得た。マウスの飼養については、九州大学遺伝子組換え実験安全管理規則並びに動物実験規則に従って実施した。

2） 臭素酸カリウム溶液の投与
$KBrO_3$ を純水に溶解し、0.2%溶液を調製後に濾過滅菌し、マウスの飲料水とした。投与法は、16 週間の自由飲水で行い、消費量については週一回モニターした。

3） 発がん実験
マウスを安楽死させた後、腸管を摘出して 10%ホルマリンを用いて固定した。その後、固定液を 70%エタノールに置換えて、腸粘膜を実体顕微鏡下で観察し、腫瘍の形成を確認した。検出した腫瘍から病理切片を作製し、病理解析を行った。

4） 腫瘍の突然変異解析
臭素酸カリウムによりマウスの腸管に誘発された腫瘍組織からゲノム DNA を抽出して、*Ctnnb1* 遺伝子を PCR で増幅した後、Direct sequencing 法を用いて変異解析を行った。

5） 統計的手法
すべての測定値について平均値と標準偏差を求めた。

（倫理面への配慮）
動物実験の実施に際しては、九州大学動物実験委員会の承認を得て、九州大学動物実験規則に従い、実験動物に対する動物愛護に関して十分配慮して行った。

Ｃ．研究結果

臭素酸カリウム誘発消化管発がん
これまでの *Mutyh* 遺伝子欠損マウスを用いた 0.2%の $KBrO_3$ 溶液を 16 週間連続飲水投与した誘発消化管発がん実験では、野生型マウスと *Mutyh* 遺伝子欠損マウスの十二指腸・空腸に 1 個体当りそれぞれ平均 0.8 および 51.0 個の上皮性腫瘍の発生を認めた。今回、$KBrO_3$ を投与した野生型マウス 5 匹および *Msh2* 遺伝子欠損マウス 4 匹に生じた 1 個体当りの平均腫瘍数は、それぞれ 1.2±0.98，27.3±1.09 であった。非投与群の *Msh2* 遺伝子欠損マウスには 1 個体当り平均 1.2±0.75 個の腫瘍が観察された。これらの $KBrO_3$ 投与で誘発された腫瘍は、前回同様すべて十二指腸および空腸の口側領域に生じていた。病理解析の結果、これらの腫瘍は 1 例を除き全てヴィエナ分類のカテゴリー4 と診断された（1 例はカテゴリー3 に分類された）。*Msh2* 遺伝子欠損マウスに誘発された腫瘍組織における *Ctnnb1* 遺伝子の解析は現在まだ進行中である。

Ｄ．考察

ミスマッチ修復遺伝子が欠損するとヒトでは HNPCC が発生する。私達はこれまで

に、通常の飼育条件下で育てた野生型マウスの腸管上皮細胞では、加齢と共に酸化ストレスに起因する突然変異が蓄積することを見いだし、他の臓器に比べて消化管は酸化ストレスの負荷が大きい器官であることが推測された。また、酸化 DNA 損傷による突然変異を抑制する *MUTYH* 遺伝子に欠損を持つ MAP 患者では、酸化ストレスに起因する自然突然変異が大腸腺腫症を引き起こす。これらの発見から、ミスマッチ修復系遺伝子の欠損による HNPCC の発症には、酸化ストレスが大きく寄与していることが考えられる。今回の実験で、$KBrO_3$ の投与により酸化ストレスが付与された *Msh2* 遺伝子欠損マウスの腸管では、顕著に上皮性腫瘍の発生頻度の上昇が認められた。このことは、上記の仮説を強く支持する。MSH2 が酸化 DNA 損傷である 8-オキソグアニンの除去に関与することを示唆するデータが報告されているが、ミスマッチ修復が実際に 8-オキソグアニンの DNA からの除去に直接関わっているのか等の詳細については明らかではない。現在、*Msh2* 遺伝子欠損マウスに誘発された腫瘍での *Ctnnb1* 遺伝子の解析を行っているが、これまでの解析では、8-オキソグアニンに起因する G→T 変異は稀で、多数の G→A 変異を見いだしている。これらの結果は、ミスマッチ修復は 8-オキソグアニンの除去には直接関与していないことを示唆している可能性がある。今後さらに *Ctnnb1* 遺伝子等のがん関連遺伝子の解析やトランスジーンを用いた酸化ストレス誘発変異の詳細な解析を行うことで、ミスマッチ修復遺伝子の欠損によって引き起されるヒトの HNPCC の発がん機序の解明を進めたい。

E．結論

MSH2 は酸化ストレスに起因する発がんの抑制に重要な役割を果たしていることが明らかになった。従って、ミスマッチ修復系遺伝子の欠損によって引き起されるヒトの HNPCC 発症には酸化ストレスが要因であることが強く示唆される。

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G．知的所有権の取得状況

1. 特許取得
特になし。
2. 実用新案登録
特になし。
3. その他
特になし。

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分担研究報告書

低レベルPhIPの持続的曝露により誘発されるDDRの分子機構の解明

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研究要旨
本研究では、DDR の初期過程としての細胞周期停止に焦点を当て、ゲノム損傷初期に分解が誘発される Cdc25A と後期に分解が誘発される Cdc25B について、DDR 誘発性分解に関わる遺伝子を解明する。本年度は、Cdc25A と Cdc25B の安定性制御因子を分離・同定するために、細胞内でそれぞれのタンパク質に結合する因子を TAP 法により精製するためのプラスミド構築を行った。即ち、両 cDNA の N-末端に TAP タグとして streptavidin 結合ペプチド（SBP）とカルモジュリン結合ペプチド（CBP）をタンデムに配置した。さらに上流に Myc タグを配置した cDNA クローンを得た。これらは、Tet-ON 細胞で発現させるためのプロモーター下に置き、一過的発現により Tet 存在下で特異的に発現誘導される事を確認した。これにより、目的細胞の分離の準備が整った。

A．研究目的

本研究では、疾患モデル動物を用いた環境発がん機構解明のうち、代表的な環境発がん物質である PhIP による発がんの初期過程を明らかにする。特に、本研究では動物レベルでは解明が困難な、発がんの初期過程である DDR のうち、細胞周期停止機構の解明を目指す。そのために、ゲノム損傷を受けた細胞で、1 時間以内に分解が誘発される Cdc25A の安定性制御に関わる新規遺伝子の分離を目指す。また、対照として、遅れて分解が誘発される Cdc25B の安定性制御に関わる遺伝子についても解析する。

B．研究方法

Cdc25A と Cdc25B の分解誘発系を解明するために、Tandem Affinity Purification を行い、Cdc25A や Cdc25B と相互作用するタンパク質を精製し、質量分析により同定し、それらの中から DNA 損傷応答と関わりのある分子を解析するというアプローチをとる事とした。

Cdc25A または Cdc25B の cDNA の N-末端に streptavidin 結合ペプチド（SBP）とカルモジュリン結合ペプチド（CBP）をタンデムに配置した。さらにその上流に Myc タグを付加し、Cdc25A および Cdc25B タンパク質をウエスタンブロッティングで検出できるようにした。　これらの遺伝子は、テトラサイクリン発現誘導系で発現させる事としているため、Tet-ON 発現用プラスミドへ移した。

テトラサイクリン依存的発現は、Tet-ON/HeLa 細胞への一過的導入と培地へのテトラサイクリン添加により行った。

（倫理面への配慮）
培養細胞使用実験のため、特になし。

C．研究結果

Cdc25A または Cdc25B は SBP および CBP に対するアフィニティーカラム（Streptavidin カラムおよびカルモジュリンカラム）を用いて、タンパク質が穏和な条件で精製できるような形にしてある。

また、この Myc タグにより、抗 Myc 抗体ビーズを用いてタンパク質の精製も可能である。さらに、Myc タグの下流に TEV プロテアーゼ切断配列を挿入してあるために TEV プロテアーゼにより複合体をカラムから切り離すことによる精製も可能である。

作製されたプラスミドのヌクレオチド配列の決定および Tet-ON/HeLa 細胞への一過的導入後のテトラサイクリン添加実験により、付加されたタグの分子量に相当する程度の分子量が上昇したシグナルが、抗 Myc 抗体によりテトラサイクリン添加依存的に検出されたため、目的とするプラスミドが作製されたものと結論された。

さらにこれらの実験の過程で、現在所有の Tet-ON/HeLa 細胞はこれ以降の遺伝子発現細胞の分離やタンパク質精製のための細胞調製には不適当である事が判明したため、新たに Tet-ON 細胞を HeLaS3 から分離する事とし、現在コロニーの単離・確認を行っている。

D．考察

今回作製した TAP 用プラスミドは SBP や CBP に対するアフィニティークロマトグラフィーにより非常に穏和な条件でカラムからの溶出が可能であるため、Cdc25A/Cdc25B タンパク質と細胞内でマ

イルドな相互作用をしている因子も精製されるものと期待される。

また Myc タグにより抗 Myc 抗体カラムを用いて Myc ペプチドによる穏和な条件下での複合体の溶出も可能であるし、結果の項に記載のように TEV プロテアーゼを用いた穏和な条件でのビーズからの複合体の切り離しも可能である。

このように、本研究で作製したプラスミドを用いて、TAP により Cdc25A や Cdc25B と種々の条件下で相互作用する因子を分離する事により、DNA 損傷初期過程でこれらのタンパク質に相互作用し、分解誘発に関わる因子の探索が可能となった。

E．結論

本研究では、DDR 初期応答として細胞制御因子の分解誘発を制御する遺伝子を、siRNA や shDNA ライブラリーを細胞へ導入することにより同定するという昨年度の方針を転換し、TAP 法により Cdc25A や Cdc25B と相互作用する因子を同定し、DNA 損傷の初期応答としてこれらの安定性に関わる因子を解明することと方針転換した。

この目的で、Cdc25A/Cdc25B の N-末端側に Myc タグ配列-TEV 認識配列-SBP-CBP という TAP 用のタグ配列を付加した cDNA を作製した。これらの cDNA は Tet-ON 細胞で発現させるため、Tet-ON 応答プラスミドに導入し目的プラスミドを得た。これらを Tet-ON/HeLa 細胞へ一過的に導入することにより、テトラサイクリン特異的なタンパク質の発現を確認された事から、当初計画したプラスミドが得られた事が確認された。

今後はこのプラスミドを Tet-ON 細胞へ安定導入し、テトラサイクリン存在下で PhIP 等を処理する事により、DNA 損傷応答特異的に安定性を制御する因子の同定を行う。

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2. 学会発表

なし。

G．知的所有権の取得状況

1. 特許取得

なし。

2. 実用新案登録

なし。

3. その他

特記すべき事なし。

研究成果の刊行に関する一覧表

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Chapter XX

PolyADP-Ribosylation in Postfertilization and Genome Reprogramming: Implications for Carcinogenesis

Tomoharu Osada and Mitsuko Masutani

Additional information is available at the end of the chapter

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1. Introduction

Pottranslational modification of proteins (PTM) is involved in molecular targeting or signal transduction as a basis of a variety of biological processes of the cells. Approximately 300 PTMs are existed in the cells, some of which are supposed to have important roles in cell physiology or embryogenesis. In addition, some modifications are reciprocally interacted, which regulates gene expression or protein dynamics or processing. We focus here polyADP-ribosylation (PARylation), which is found to contribute importantly at fertilization and postfertilization development. Moreover, we discuss the possible drug discovery of ADP-ribosylation inhibitors in carcinogenesis or fertility control.

Upon fertilization, two kinds of genetic materials meet together to generate a new organism [1]. Meanwhile, the organism develops without major expression of genes from genomes. Although maternal proteins are supposed essential for the period, the details are ill understood. Transition of zygotic gene expression is also essential for further development. Some PTMs on transcriptional factors may be key regulation to express the genes which supports early development. Parental DNAs are differently regulated in the postfertilized eggs, although the biological significance is controversial. Theses implications suggest importance of PTMs on postfertilization and transition of zygotic development. Recently we found that PARylation, a PTM of protein is important for postfertilization development, which seems similar with those of carcinogenesis.

The PARylation reaction is a PTM of proteins, which is synthesized with poly(ADP-ribose) polymerase (PARP) and metabolized with poly(ADP-ribose) glyceraldehyde (PARG) [2,3]. The reciprocal regulation is speculated as a key mechanism that underlies reversible

regulation of gene expression. The poly(ADP-ribose) is generated from NAD, an energy reservoir of the cells by PARP. The PARP synthesizes poly(ADP-ribose) using NAD. PARP polymerizes ADP-ribose residues to generate poly(ADP-ribose) chains onto proteins. PARylation reaction itself is regulated by auto-PARylation of Parp1. Since the ADP-ribose residue is negatively charged, the acceptor proteins become negatively charged by the addition of ADP-ribose residues. The electrical charge-shift of the protein induced by PARylation may decrease an easy access of proteins into the DNA structure or may induce structural instability of protein-DNA interactions, because the negative charge of poly(ADP-ribose) supports its association with DNA-binding proteins, which are positively charged. The recognition of DNA sequences by protein structure may also be affected by PARylation. Upon postfertilization development, zygotic gene expression is essentially activated prior to the 4-cell cleavage stage in mice and human. Therefore, behavior of maternal molecules plays important roles at postfertilization development before zygotic gene activation. PTM of protein is supposed to contribute to protein dynamics at postfertilization development. Pharmacological blockage of PARylation revealed defects of postfertilization development in mice [4]. The data raise a question regarding the roles of PARylation at fertilization development. A wealth of study revealed that NAD is rich in eggs and rapidly degraded upon fertilization in Xenopus larvae. Parp activation at fertilization may contribute to consumption of NAD, which may bring a plausible explanation towards the uncovered subject. Postfertilization development is specific biological window to highlight the significance of PTM, because transcription is minor mechanisms for the organisms at the period. In this chapter, we discuss the roles of PARylation in eggs and the function of postfertilization development. Further we discuss the possible implications to drug discovery focusing on PARylation regulation. We focus on polyADP-ribosylation because we previously showed that no fertilized eggs were obtained by pharmacological blockage of PARylation by a PARP inhibitor, PJ-34 [4]. Other inhibitors of PARylation showed similar results. Therefore, a hypothesis was raised that posttranslational regulation is key regulation of maternal genetic materials at fertilization because no robust transcription is occurred at the beginning of life.

2. PARylation in postfertilization development

Recent research showed that pharmacological blockage of PARylation leads to defects in pronuclear fusion during postfertilization in mice. Based on the observations, several approaches were achieved to investigate the roles of PARylation in vivo and mechanisms of reprogramming applicable for regenerative medicines or elucidation of human diseases including carcinogenesis.

Based on our observations regarding the disorganized microtubule assembly in oocytes by PARylation inhibitors, polyADP-ribosylated proteins of oocytes and postfertilized eggs were searched. We found that the tubulins (α1c, β2c) [4] and glutathione *S*-transferees μ5 (GSTμ5) (Osada et al., unpublished data) was highly polyADP-ribosylated after fertilization.

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Because PARylation is known as a regulatory machinery of DNA surveillance system, this data provided us with a novel sight of view on the roles of PARylation regarding cell signaling and metabolisms. As a major upstream signaling cascade after sperm entry, MAP-kinase (MAPK) signaling has been postulated as the key regulatory mechanism for pronucleus formation, and Parp1 is reported to be involved in the regulation of MAPK signaling [5]. Our data suggest that Parp1 is a novel nuclear component for PPN (Pseudo pronuclei) formation, which may be mediated by MAPK signaling. Phosphorylation level of Erk was decreased in the MII phase of Parp-1 null oocyte. Because the downregulation of Erk phosphorylation is known to disturb microtubule organization, these results raise a possibility that the transient administration of a Parp inhibitor may block the early phase of post-fertilization process.

Second, we focused on the roles of polyADP-ribosylation on epigenetic regulation. Accumulating study suggests that PARylation has a fundamental role in transcription as well as DNA surveillance, which mediates topological changes of the DNA structure [6,7]. We first examined the effects of PARylation on chromatin modification during postfertilization development. An immunofluorescence study using histone acetylation and methylation antibodies were initially carried out. Upregulation of dimethyl-H3k4 in the PARylation-inhibited embryos at 6 hpf, and downregulation of dimethyl-H3K9 and dimethyl-H3K27 at 15 hpf were observed (unpublished data, Osada et al.). Recently, functional links between histone methylation and DNA methylation have been elucidated. Based on our observations of distinct effects of PARylation inhibition on histone modification regulation, we next sought for the roles of PARylation in DNA methylation. Global methylation of the maternal genome is maintained and sperm DNA is quickly demethylated during the postfertilization period [8]. The low immunoreactivity to the MetC antibody in the PARylation inhibited embryos was observed and it persisted at least until 15 hpf (unpublished data, Osada et al.). The data suggest that epigenetic regulation is asymmetrically activated during postfertilization development, although gene expression is not activated. Therefore, PARylation affects the integrity of epigenetic regulation of oocytes and postfertilized eggs (Figure 1).

An additional implication of this finding is that PARylation regulates the epigenetic nonequivalence of the pronuclei in mice. The molecular mechanisms regulating the asymmetric DNA methylation of one-cell embryos are largely unknown, and we showed that PARylation is involved in the epigenetic regulation of the mouse early development. PGC7/Stella-deficient eggs show similar defects in the protection of female pronuclei from DNA demethylation [9]. The nucleo-cytoplasmic transport of the responsible proteins appears to be important for the regulation of DNA methylation. The role of PARylation in DNA methyltransferase (DMNT) regulation is yet to be fully elucidated, although the interaction of DNMT1 with PARP1 and the indirect repression of DNMT1 activity by interacting with PAR have been suggested [10]. Based on our data showing an association between PARylation or PARP and histone modification, it is speculated that PARylation

may regulate the accessibility of DNMT or demethylase to DNA, in a manner mediated by chromatin remodeling. Our study provides a novel avenue for better understanding of establishment of chromatin organization through the histone codes and DNA methylation that may underlie at the beginning of zygotic development.

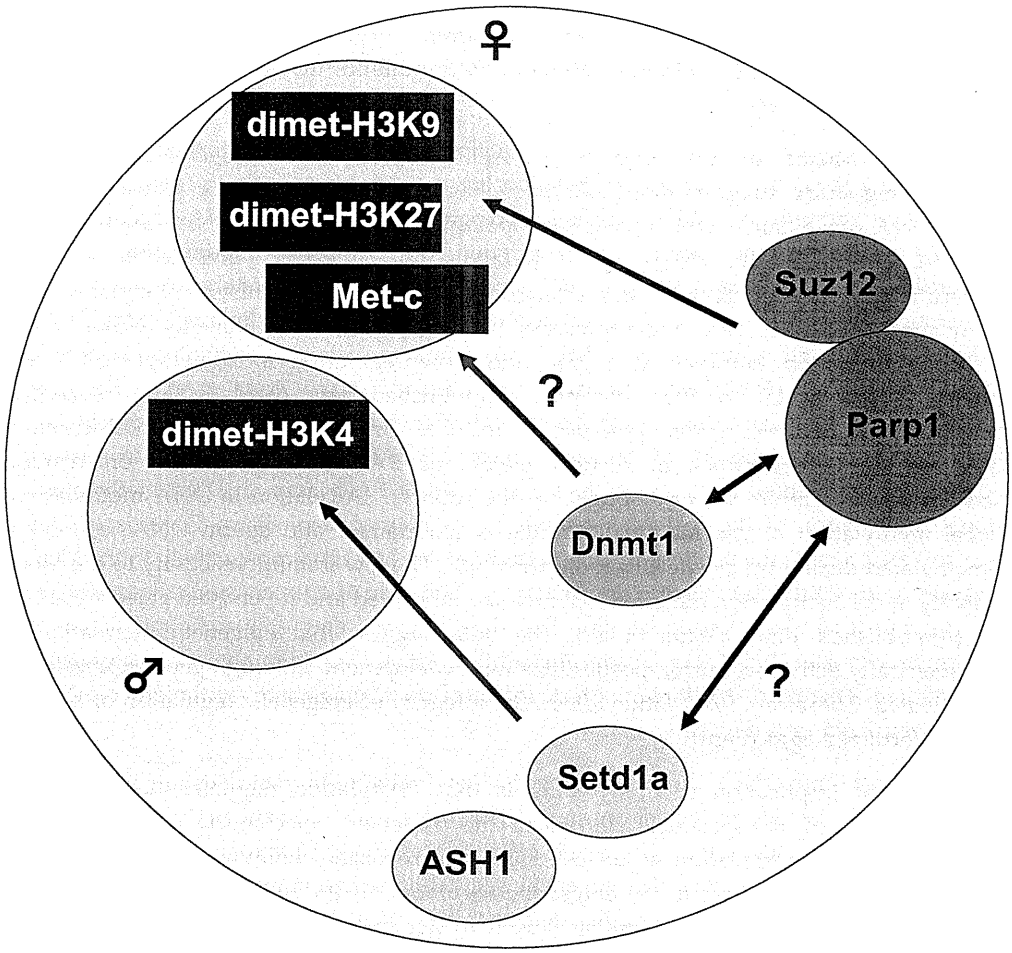

Figure 1. Scheme of putative roles of PARylation in the chromatin dynamics of one-cell embryos. In the one-cell embryos (yellow circle), PARP1 may interact with histone modifying enzymes including Suz12, that might affect the asymmetric regulation of histone modifications in the pronuclei (male pronucleus in blue circle and female pronucleus in green).

To examine whether there is a direct or indirect linkage of epigenetic regulation with PARylation, we screened molecules that interacts with Parp1. To elucidate the molecular basis of the effect of PARylation on histone modification, we performed yeast two-hybrid screening using the bait vector carrying N-terminal and automodification domain. The Suz12, a histone methyltransferase, was identified as a candidate molecule interacting with Parp1 (unpublished data, Osada et al.). Suz12 is a component of PRC2/3/4 (Polycomb Repressive Complex 2/3/4), which regulates the repressive status of transcription by the methylation of histone H3K9 and H3K27. Our findings support the idea that Parp1 acts as a regulatory scaffold for the access of PRC2/3 to target DNA, because none of the components of the PRC2/3/4 complexes are DNA binding proteins. OCT4, bidirectional transcriptional regulator, is suggested to play a role of transcriptional regulation by recruiting PRC2/3/4 to the target genes, which are indispensable for the embryonic development. Modification of PARylation triggers loosening of chromatin structure, which may enable the access of transcriptional factors to DNA duplex structure.

Third, as shown in Figure 2, defects in pronuclear formation by inhibition of PARylation raise a possibility that PARylation is involved in laminar formation of pronuclei [4]. In fact, HP1, an anchor protein of lamina and heterochromatin is polyADP-ribosylated [11]. Our findings showed prolonged presence of lamin-A/C, a core protein of lamina by PARP inhibitor PJ-34 treated eggs. Interestingly, lamin-A/C is predominantly existed in undifferentiated cells. Lamin-A/C is known as a marker of carcinogenesis [4]. Our observation provided a new insight into regulatory machinery of laminar formation in pluripotent cells by PARylation. Our initial data suggest that cell-cycle is not disturbed when embryos were subjected to treatment of PARP inhibitor.

During the first cell cycle of mouse embryos, a few genes are transcribed mainly from paternal genome. Inhibition of transcription during one-cell embryos by RNA polymerase inhibitors showed dispensable roles of transcription in normal development. Zygotic gene activation is required for progression from 2-cell to 4-cell embryos. These indicate that posttranslational regulation of protein should act as a stem mechanism of the development of one-cell embryos. Upon fertilization, highly compacted chromatin of gametes was acutely decondensed to form pronuclei (PN) within a few hours. Protamines of sperm chromatin are replaced by maternal histone H1 during this process, which may be associated with global hypomethylation of sperm-derived PN. In contrast, maternal chromatin arrested at metaphase II progresses rapidly into G1 phase and subsequently forms the female PN. DNA synthesis from paternal genome is preceded to that from maternal DNA. A minor transcription is activated solely from male PN. This evidence suggests that the requirement for the posttranslational regulations of parental genomes before mingling of both gamete DNA to begin the proper zygotic development. Of posttranslational modification of proteins, we examined here the effects of PARylation during the first cell cycle of mouse embryos. Metabolism of NAD, which is the substrate of PARPs, is acutely activated upon fertilization. Further analysis will elucidate the biological functions of PARylation upon fertilization and downstream target molecules of them.

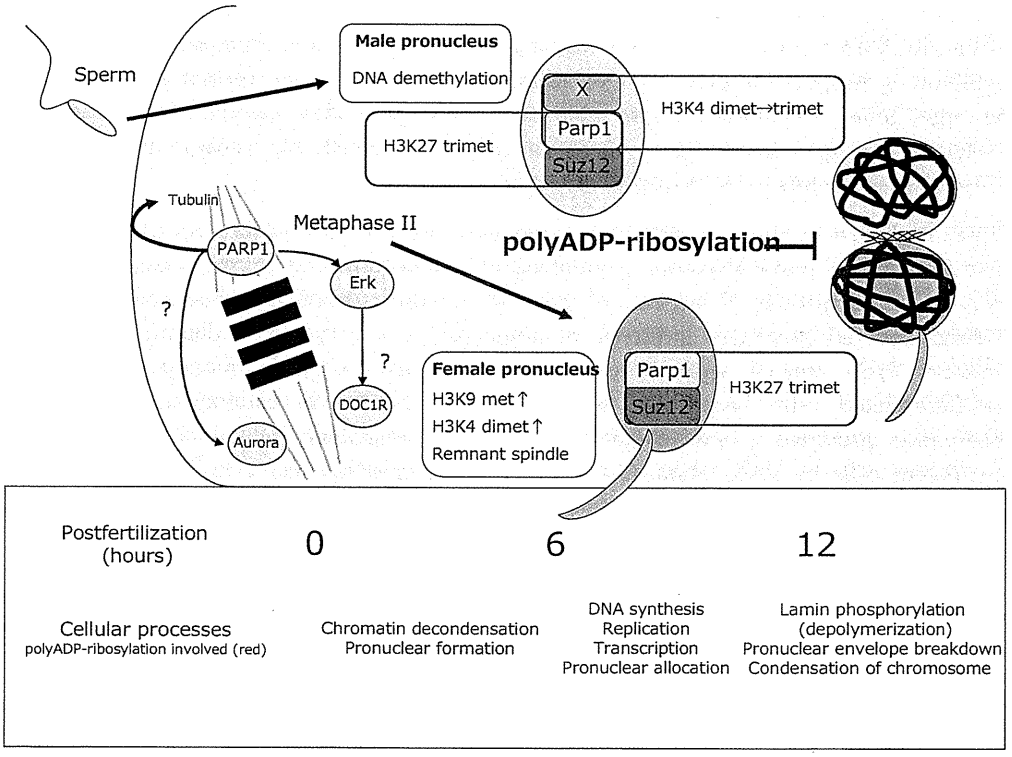

Figure 2. Model for regulation of pre- and postfertilization process by PARylation. Biological processes occurred during pre- and postfertilization were shown. Putative correlation with polyADP-ribosylation were indicated in red.

3. Implication towards reprogramming

In 1997, the first cloned animals were generated [12]. Cytogenetic analyses have shown that karyotypic aberrations occur in cloned embryos during the first mitotic cleavage. Epigenetic errors in cloned animals are also argued to be the major reason for the limited success rates of cloned animal births. However, whether genome-wide chromatin remodeling during nuclear reprogramming causes DNA damage, or whether defects in DNA repair cause the inefficiency of cloned animal births have not been investigated. Recently, it was reported that double strand break (DSB)–mediated chromatin remodeling regulates transcription, and that Parp1 is critically involved in this process. Various roles for Parp1 have been described, including in the chromatin remodeling involved in transcriptional regulation [2,3]. The roles of Parp1 in NT-embryo development in the contexts of DNA repair and chromatin remodeling was examined. To do this, we used *Parp1*-null mutant cells as recipient oocytes and as a source of donor nuclei.

We observed that the activation process in NT eggs was enhanced in *Parp1*–null NT embryos, although some genomic instability was observed (Osada T., unpublished data). Dynamic changes in histone acetylation and methylation were induced under *Parp-1* deficiency in NT embryos (Figure 3). The lack of Parp1 may thus facilitate chromatin condensation or transcriptional silencing. Parp1 is involved in both the regulation of DNA strand break repair and the epigenetic control of gene expression. Our findings suggest that Parp1 is important for chromatin remodeling possibly through histone modification during the nuclear reprogramming of NT embryos. The phosphorylation of histone H2AX at DSBs is believed to be crucial for the recognition and repair of DNA damage. The foci-like presence of γH2AX in the NT embryos implied that DNA repair was taking place in the PNN. H2AX is a direct target of ATM kinase, which is expressed at the spindle of MII oocytes, and Parp1 interacts with ATM. The prolongation of the γH2AX foci in the *Parp1*$^{-/-}$ NT embryos could therefore be related to a delay in repairing DNA strand breaks, as is the case with *Parp1*$^{-/-}$ MEF and ES cells, which show prolonged γH2AX foci after DNA damage induced by neocartinostatin. In *Parp1*$^{-/-}$ NT embryos, mitotic arrest of embryogenesis at the 2-4-cell stage was frequently observed, accompanied by polynucleated blastomeres. Since Parp1 is located in centrosomes as well as in nuclei, the lack of Parp1 may have disturbed the normal cell division cycle and the synchronous pattern of cell division among the blastomeres. Transient Parp-1 functional inhibition may be therefore useful to improve the efficiency of NT by modulating the dynamic organization of chromatin without causing genomic instability.

4. Implication towards human diseases

PARylation has been known to possess important roles in carcinogenesis. PARP mainly affects DNA surveillance system in the cells, which deficit increases the risk of carcinogenesis. In addition, recent study showed a functional relationship between PARP

and MAPK signaling, known as a major intracellular signaling of transformation into cancer cells. Although morphological change of nuclear envelope has been known in cancer cells, the molecular mechanism of the processes is not well understood. Recent studies revealed molecular signaling of laminar formation is altered during carcinogenesis. Our study revealed that PARylation is involved in these molecular events. Oocytes are capable of being isolated without other cell types by adding hyarulonidase, which is far easier compared with the difficulty in homogenous isolation of purified cancer cells. Our data suggest oocytes provide with a unique biological window for elucidating the mechanism of PARylation in carcinogenesis. Further comparative analysis between cancer cells and oocytes may highlight the uncovered mechanisms underlying the carcinogenesis processes.

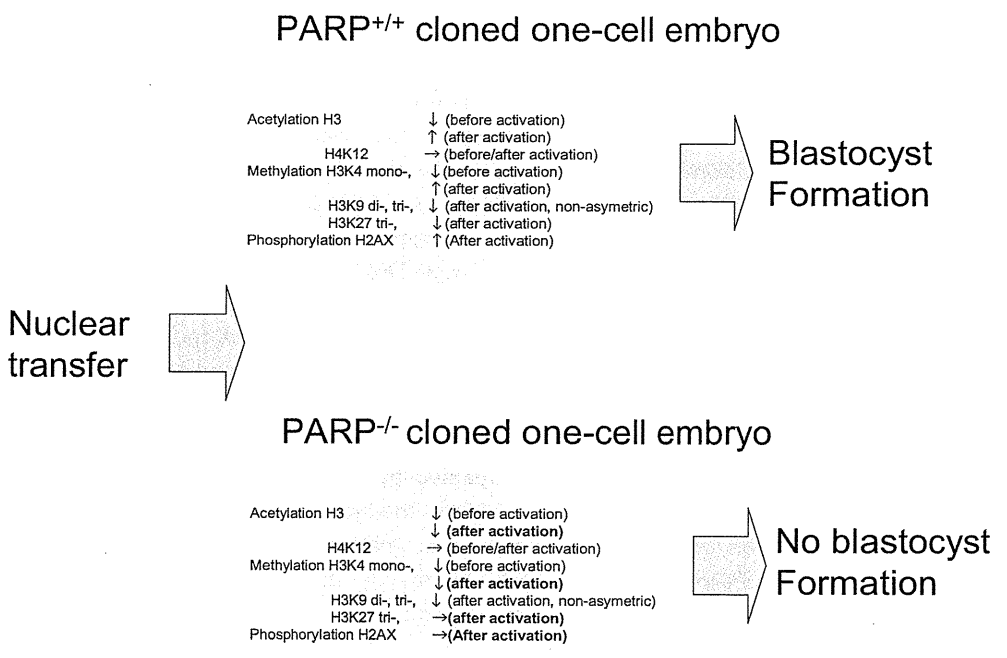

Figure 3. The scheme of histone modification and the effects of *Parp1* deficiency during the first cleavage of NT embryogenesis. Histone modifications of the one-cell reconstructed oocytes were indicated in *Parp*$^{+/+}$, and those which *Parp1* deficiency influences were indicated in *Parp*$^{-/-}$ in red.

We also described here that PARylation is a novel target of anti-conception. Inhibition of PARylation may easy to be handled, because exposure of an inhibitor to oocytes is effective enough to stop fertilization. Further extensive analysis should be carried out regarding the optimized dose of inhibitors and non-toxic dose on other tissues. Biological phenomena, in which PARylation is involved, are variable and its inhibitors could serve as possible pharmaceutical targets including inflammation or brain injury as well as carcinogenesis and reproduction as discussed in this chapter. Further basic investigation of the roles of PARylation in the cells will broaden the view for the understanding the embryogenesis and proof of mechanism of human diseases for drug discovery.

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