Disregulation process of cancer cell proliferation

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Disregulation Process of Cancer Cell Proliferation

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INTRODUCTION

Sequential process in one cell cycle persist through certain mechanism that analog to interrelated biochemical pathway. Indeed, every phase of this process is regulated as if it is regulated by internal clock. Hence, it always persists on time. The cell cycle clock is periodic activation of cyclin-Cdk complex. It is tightly controlled by certain point in cell cycle, known as cell cycle decision points or checkpoints. At that checkpoints, the cell cycle may be temporarily terminated (reversible), when it is needed, as a response to external or internal signal. Various growth factors may stimulate cell so that it is able to complete cell cycle though one or more checkpoints and it may proliferate and differentiate in balance appropriate to its necessity. Other factors that important in this balance are growth inhibitory factors such as transforming growth factor β (TGFβ) by enhancing the function of Cdk inhibitor.

Various inventions of proto-oncogene and tumor-suppressor genes which are important in normal cell cycle, have enhance our knowledge about correlation between over activation of oncopgenic and lost function of suppressor genes with uncontrolled cell cycle and abnormal genes content in a cell that undergone transformation. Most of oncogenes and tumor suppressor genes normally regulate cell cycle through direct intervention. Mutation that causes amplification of oncogenes function has caused component activation – the component (such as activation of ras oncogenes), which is effective through a signal cascade that correlate between original signal and growth receptor with receiving genes of growth signal so that cell cleavage is occurred. In other hand, mutation on suppressor genes has caused losing of suppression function of cell growth. Therefore major component of cell cycle regulation machine and signal transduction pathway, which correlates the growth factor and that machine are the most-happened oncogenic target lesion.

NORMAL CELL CYCLE

Every cell cycle consist of 4 serial phases which is tightly controlled, that are G1 (gap 1), S (synthesis of DNA), G2 (gap 2) and M (mitosis / meiosis) (figure 1). DNA replication is in S phase; chromosome separation (caryokinesis) and cell cleavage are in M phase, while G1 and G2 are growth phase. Inactive cell (un-growing cells) is in G0 phase, which is resting phase; in mammalian, growth controlling largely occurred in G1. Determining factors of growth acceleration are factors that cause the cell arrested at G0 or out from G0 and entering G1, and there is variation of time. The DNA content of G0 is equal to G1 phase that is 2N. S phase persist about 12-24 hours in mammalian. Specific chromosome part replicates continuously in certain time so that the DNA content reach 4N. G2 phase usually occur for 3 hours. This phase stop when interaction of cyclin and other factor begin in prophase of M phase. The time needed to accomplish one cell cycle range from 2 – 4 days.

Controlling of mammalian cell cycle largely occur at G1 phase. External signal – such as given by growth factors, is needed so that the cell exit and it has resting phase (G0), then entering the G1 and S, that DNA synthesis occurred. During G1, every cell responsive to and depend on various extra-cellular stimulus, so that the G1 is regarded as the most important part of growth controlling system. In contrast, at certain condition we need some effort so that the cell constantly in G0 phase, for example bone marrow stem-cell at still condition. Even
the function of c-fos in some of cell type is to enhance cell proliferation, but in the stem cell of bone marrow it has other impact. In this cell, over expression of c-fos in G0 phase will prevent hemopoetic stem cell entering G1 phase. The mechanism of this different impact is unknown yet.

Some sequential process must occur before the cell of G0 phase entering G1 phase at restriction point, that are: a) binding of one or more growth factor on specific receptor; b) transmission of growth factor signal on nucleus; c) gene activation to form protein i.e. fos, myc, Jun, etc; d) protein binding on DNA, then it regulates other genes to produce protein as needed in order to continue or terminate cell cycle. Hence, the cell cycle is regulated by external signal and internal control system. The G1 pathway needs activation of various cyclin-dependent kinase in sequence and inhibition of Rb suppressor genes through phosphorilation. The cell in cycle must activate serial of positive factor and suppress various inhibitor functions.

DNA replication and mitosis must occur regularly in serial. Every cell must be able to determine whether a phase and cell cycle have completed before it advances to the next phase. For example, the cell must monitor whether S phase has completed before it is ready for mitosis. If there is any defect of DNA replication, normal cell will stop at S phase and will not continue to M phase. This regulation system protect cell so that it will not proliferate abnormal cells as though if there is any damage of DNA, the cell cycle will temporarily stop to give a chance of DNA reconstruction before it continues to the next phases, or that cell will have programmed death or apoptosis. So, the cell cycle regulate duplication of genetic information and the duplicated-chromosome separation accurately to their inherited cells. Checkpoints is a pause of one cycle, by then there is monitoring of duplication accuracy and chromosome regulation. A this checkpoint, there is editing or reparation of genetic information if it is needed, so that every inherited cells have a complete genetic information instrument that is identical to the original cell.2

Growth Factor (GF) and Growth Factor Receptor (GFR)

Some of normal cell proliferation and differentiation process are regulated by extra-cellular factor, including various growth factors (GF) that has polypeptide form, which induce proliferation of the right cell-target. Platelet derived growth factor (PDGF) is the main growth factor of fibroblast. The active PDGF consists of 2 kind of peptide with 40% identical amino-regulation; each has alpha and beta chain. The genes that code two kinds of peptides are in different chromosome. The active PDGF molecule is a dimer, which is mutually related through bi-sulfide bound; this dimer may consist of alpha and beta chain (hetero-dimer). Some studies reveal that only cells that responsive to PDGF-β and expressed PDGF on its surface, which easily transformed.

Other GF group is fibroblast growth factor (FGF) which is consist of acidic FGF (FGF1), basic FGF (FGF2), int2 product (FGF3), hst product (FGF4), and FGF5. FGF6 and keratinocyte growth factor (KGF) as mitogene of epithelial cells are included in this group. Epidermal growth factor (EGF) stimulates proliferation of various kinds of cell. Other growth factors, which are similar to EGF, are also known, such as transforming growth factor-alpha (TGF-α), amphiregulain and others. All GF of this group stimulate cell proliferation through binding with EGFR, which is product and proto-oncogene of erbB. This GFR group is one of GFR that embedded in cell membrane. TGF-α and EGF have capability to transform cell, and over expression of this GF may cause tumor growth.
Proliferation and differentiation of hemopoetic cell are also controlled by serial of polypeptide, which cause specific impact on different kind of cell. There are four important type of hemopoetic GF, i.e. IL2 (T cell growth factor), IL3 (multipotential colony stimulating factor), GM-CSF and CSF-I. CSF1 is also known as macrophage colony stimulating factor (M-CSF), which is synthesized by activated monocyte and macrophage, and also by fibroblast and other mesenchymal cell. CSF1 is assumed to be involved in MDS and AML development.

Signal Transduction

The results of various genetic studies reveal that there is important gene, which is function as regulator of cell cycle universally. First, they found cdc2 in yeast, which codes protein 34 kDa, which is called as serinethreonine protein kinase p34cdk2. The next studies reveal some homologue and cdc2, which now known as cyclin dependent kinase (cdk), which its activity is regulated by cyclin. Cyclin is categorized in 2 functional group, that is cyclin which is effective in G2/M limit (cyclin B1 and B2), and cyclin which is effective in G1/S limit (cyclin C, D and E), while cyclin A is an exception which is effective through S up to M phase. Cyclin B is synthesized in G2 phase and has maximal level during mitosis. Cyclin B is needed to enter mitosis phases while cyclin degradation is needed to exit the mitosis phase. Cyclin C, D and E is especially expressed in G1 phase. It co-operate with cdk2, cyclin E – which strongly correlated to suppressor gene product Rb (p107) – and E2F transcription factor. Cyclin cdk4 complex has specific function in phosphorylation of Rb gene product.

Besides the cyclin-cdk that basically regulates persistence of cell cycle, there are other factors that prevent excessive growth. These factors are cyclin/cdk kinase inhibitor (for example p21/p16 and p27). Protein p21 is protein that inhibits the activation of cyclin/cdk and it is gene product, which is activated by p53. Brgarolas et al in their study proved that p21 is cdk2 gene regulator that essential to control cell proliferation with Rb defect. The G phase of cell with p21 defect is shorter than p21 wild type. Even though, the short time of this G1 phase does not cause shorten time cycle in general. From their study, they conclude that growth control mainly occurs in G1 phase and p21 and Rb are important factor in that growth controlling system. The researchers also have proven that if there is any defect on Rb, cdk2 has a role as “gatekeeper”, which determine the growth control. Activation of cdk4 and cdk2 pathway continuously have adequate to cause uncontrolled proliferation and cause the cells unable to response stimulus to stop cell cycle. Beside it may stop cell cycle through cyclin/cdk pathway, p21 may also directly prevent DNA replication by inhibiting PCNA activity. PCNA is a protein that needed in DNA replication by polymerase d and e. The PCNA activity may inhibit by binding PCNA on PCNA-binding region on p21 by directly interacted between p21 with PCNA, and that inhibition may be eliminated if there is excessive PCNA.

Protein p21, p16, and p27 mediate growth termination when there is no growth factor, if there is stimulation by negative regulator or if there is DNA-damaging substances.

DISREGULATION OF CELL CYCLE IN CANCER

The ability to autonomic proliferate or uncontrolled are one of alteration of transformed cell phenotype. The cell, which experienced transformation by excessive oncogenes expression or deactivation of suppressor genes do not depend on extra-cellular signal to proliferate. It is able to continuously proliferate even there is no growth factor to stimulate it, because it is able to stimulate itself (autocrine stimulation). Defect of cyclin-cdk system, especially on p34cdk2 in S phase may cause repeated DNA replication one or more in single S phase before the cell cycle enter the next phase with the effect that abnormal content of DNA or known as aneuploidi. The phenotype alteration on cancer cell is growth ability without adhesion to extra-cellular matrix (anchorage independent growth).

Autocrine Stimulation on Tumorigenation

Various studies reveal concept that production of growth factor and expression of receptor of related growth factor that persist continuously in the same cell may cause self-stimulation. This concept is introduced base on auto-stimulation pathway, which is shown in the study of epidermal growth factor receptor (EGFR) activation in cell culture. Then, other studies are also supporting that concept and reveals that auto-production of growth factor, which is important in cell proliferation, produce a mechanism that enable a cell to
proliferate without stimulation of external growth factor and it may enable the uncontrolled growth.\textsuperscript{13}

Another important evidence is expression of abnormal growth factor may stimulate cancer growth. This is obtained from studies about v-sis oncogenes. This oncogene is component that involved in transformation of simian sarcoma virus and code variation form of PDGF-β. Normally, PDGF is a monomer molecule but because of mutation on v-sis oncogene, the transformed cell will express dimeric growth factor, with similar structure and function to monomer PDGF. Hence, this PDGF is able to stimulate PDGF receptor, which is expressed by the same cell. In endocrine or paracrine stimulation, growth factor (GF) is secreted by certain cell type and bind GFR on other cell type and also stimulate it as target cell. In autocrine stimulation, GF is produced by cell that also has or expressed GF receptor. Hence, it is responsive to the self-produced GF.

Nevertheless, there is may be direct stimulation of transcription factor in nucleus by the growth factor, without stimulation through receptor. This will cause continuous stimulation cycle, resulting malignant transformation. PDGF production by the transformed cell and follow by continuous stimulation of PDGF receptor in the same cell will result as proliferated cell because it is stimulated by the self-produced substance (autocrine stimulation). (Figure 3)

Because growth factor (GF) and its receptor (GFR) are produced by the same cell, there should be intra-cellular GF-GFR binding before that factor were expressed on cell surface (intracrine).\textsuperscript{13}

### Abnormal Activation of Growth Factor Receptor (GFR)

Oncogenic mutation frequently occurs on genes that code the growth factor receptor. Oncogenic mutation causes code alteration of those genes so that abnormal protein is produced. One of important abnormality is that it is always expressed at activated condition.\textsuperscript{14}

One of important receptor in growing process is tyrosine-kinase protein, one of important protein group, which is related to activity of kinase receptor. It is a transcription factor group, which contain SH2 domain, and is controlled through phosphorilation. This protein group is known as STAT (signal transducers and activators of transcription). Phosphorilation activates the transcription factor of STAT and enhances its translocation to the nucleus, where it has function as direct activator of gene expression.\textsuperscript{7} Figure 4 shows activation of STAT transcription factor. Oncogenic mutation, which codes PTK may result abnormal PTH in such manner, so that it is in continuous activated condition, even there is no ligand binding on it.

Variation of abnormal receptor may be formed because of interaction with retrovirus, mutation or deletion of gene, chromosome rearrangement or gene amplification. The mutant and c-erbB gene form may result PTK receptor that has not had any extra cellular domain (Figure 5). The mutant and neu gene form may result PTK trans-membrane domain in dimer form, so that it is similar to activated PTK form (Figure 6).\textsuperscript{14}

![Figure 3. Autocrine Growth (Right)](image)

![Figure 4. Activation of STAT Transcription Factor](image)

![Figure 5. Mechanism of Erbb Oncogene Activation. Activity of EGF (Erbb Proto-Oncogene Protein) Tyrosine-Kinase Receptor is Controlled by EGF Binding. In Contrast Erbb-Oncogene Kinase is Continuously Active](image)
GFR abnormality may result from mutation or amplification. Many of malignancy over express the GFR on their surfaces and this over expression frequently accompanied by over production of TGF-a, a growth factor. Various clinical studies prove that there is correlation between over expression of EGFR and TOE-α production with bad prognosis and bad therapy response.\(^{13}\)

Figure 6. Oncogenic Mutation of PTK Receptor Produces Receptor with Abnormal Trans-membrane Structure, Which is Locked in Active Condition Oncogenic Mutation of PTK Receptor

Table 1. Different Proliferation Process of Normal Cell and Cancer Cell or Transformed Cell

<table>
<thead>
<tr>
<th>Normal Cell</th>
<th>Cancer Cell</th>
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<tbody>
<tr>
<td>Need external Growth Factor (GF) to activate Growth Factor Receptor (GFR)</td>
<td>Frequently able to synthesize and secrete GF, which stimulates self-growth (autocrine stimulation)</td>
</tr>
<tr>
<td>Normal GFR must first bound to GF so that it may release the growth signal</td>
<td>Abnormal GFR expression may release signal that first bound to GF</td>
</tr>
<tr>
<td>Activated GFR activates the cascade of signal transduction in cytoplasm</td>
<td>Abnormal transducer in cascade system may release growth signal without being activated by GFR</td>
</tr>
<tr>
<td>Protein activation which regulates transcription of growth regulator genes needs stimulation through cytoplasmic instruction that derived from activated GFR</td>
<td>The abnormal transcription regulator gene may activate transcription without any cytoplasmic instruction that derived from GFR</td>
</tr>
</tbody>
</table>

Activation of Abnormal Signal Transduction Protein

Oncogenic mutation may also result protein that is coded as abnormal. One of its abnormality of that protein that it is always at activated condition, even there is no external mitogenic signal. Expression of signal transduction protein at activated state as mentioned is interpreted by cell as mitogenic signal, resulting uncontrolled proliferated cell. The main mitogenic pathway of various kind of cell is ras activation. The normal ras protein will be activated if it is bind to GTP, but it will become immediately inactive by hydrolysis of GTP into GDP. In mutation of ras gene, ras protein persists in bind condition with GTP, resulting continuous activated condition of it.\(^{2,14}\)

Figure 7. The Role of Adaptor Protein in Regulation of Ras Activation Cell Membrane

Cyclin is protein group that has function as positive regulator in signal transduction pathway. Over expression and cyclin may also cause uncontrolled proliferation. Some evidence shows that cyclin D is the main onco-protein, which is involved in tumor genesis. First, the isoform cyclin D, through it’s binding with cyclin dependent kinase Cdk4 and Cdk 6, is needed for Rb phosphorilation. It means that it is needed to continue the G1 phase. Cdk4/6-cyclinD-Rb pathway is the most interrupted pathway in various type of cancer. Growth factor, such as macrophage colony stimulating factor, induce expression of cyclin D if those factors is added on cell in G0 phase. Therefore we assume that over expression of cyclin D will facilitate the G1 phase. Alteration of chromosome structure that it usually appears in various type of tumor has proven that it was occurred because of increase of cyclin D1 gene transcription. It proves that cyclin D1 was expressed abnormally on relevant tumor and it will shorten the G1 phase, so that we assume that it is involved in tumor genesis process\(^{14}\). Recently, there is successful clone of other cyclin type, that is cyclin A1. The function of cyclin A1 in regulating cell growth is not completely known, but there is evidence that cyclin A1 is involved in mitotic phase and that it has interaction with other cell cycle regulator, such as interaction with E2F-1 and Rb. In some of leukemia, over expression of cyclin A1 has been proved to lock the cell growth and differentiation at mieloblast and promiclocyte stadium.\(^{15}\)

The cell growth, besides being regulated by cyclin/ cyclin dependent kinase, it is also controlled by various negative regulator, such as protein Rb and p21, which is transcripted by p53. Individually, each of negative regulators has significant effect in regulating the G1 phase. P21 protein has been known to have strong
affinity against cyclin E/cdk2 complex, and over 95% of active cdk2 in diploid fibroblast cell is bound to p21. In order that G1 could occur, pRb, which is suppressive to the cell growth, was firstly inactivated by cyclin D/cdk4 through phosphorylation. Even the wild type Rb cell is successfully affected by cyclin/cdk4 activity; cell with deficiency of pRb is not affected by that activity. Mutation of losing function of both negative regulators, separately, or together, will cause losing of suppression function. Hence, there is uncontrolled cell proliferation and anchorage independent growth. It is proven by Brugarolas et al in their study that some of them are shown in figures.

![Figure 8](image_url)

Figure 8. The Nature of Set Growth with p21 and Rb Wild Type, p21-/-, Rb-/- and p21-/-, Rb-/- in Limited Growth Factor Environment (FCS 0.1%)

Figure 8 shows that the cell amount with defect of p21 and Rb are larger than cell with p21 and Rb wild type. Nevertheless, there is limited concentration of growth factor. Moreover, cell with defect of both gene will grow without any control. From the mentioned discussion, it is clear that tumor has autonomic proliferation, and it is not depend on inhibition signal. It is also proliferating even there is no growth signal; it means that tumor is not able to integrate extra cellular signal and cell cycle regulator machine. This defect has multilevel site, i.e. on receptor of cell surface, on the pathway of signal transduction and on cell cycle regulator. And various factors that have important role in G1 phase, we assume that p21 and Rb are important component in growth regulation pathway. There is evidence that in Rb defect, cdk2 may have function as gatekeeper, which controls growth up to certain level, and continuous activation of cdk4 and cdk2 is enough to cause uncontrolled cell proliferation and unable to stop the growth.9

CANCER CYTOKINETIC AND ITS MEASUREMENT

We should know that normal ability of cell proliferation is different for every tissue type. Cell or tissue that has fast proliferation are: bone marrow, mucosa of gastrointestinal tract, ovarium, testis, hair follicle; and cell or tissue that has slow proliferation are: lungs, liver, kidney, endocrine glands, endothelial of blood vessel, and the cell or tissue that has not proliferated are muscles, bone, cartilage and nerves. This normal cytokinetic nature may alter in cancer.

Cancer cytokinetic is important because cancer growth, invasion and metastasis are very depending on reproduction of cancer cell. Cytokinetic also determine the prognosis and therapeutic design.16,17

Non-proliferative cells consist of 3 compartments. Various high-differentiated cells such as neuron cells are in immortal non-proliferative fraction. Most of differentiated cell is not proliferate, but it has certain age; this cell group is known as mortal non-proliferative fraction. The other non-proliferative compartments are cells of unstable non-proliferative fraction; usually it exists in G0 phase that sometimes enters G1 phase, if it has been stimulated. Including in this group is bone marrow stem cell. The death cell in any phase is called as loss fraction. Tumor with a large loss fraction, clinically appear as slow-progress tumor. This phenomenon is revealed in Berges et al studies on prostate cancer. They found that in transformation of prostate gland cells into prostate cancer cell, first there was unbalanced increase between proliferating cells and the death cells resulting fast growth. In advanced stadium, the amount of death cell is larger so that it seems as if tumor growth was stopped even it has increase proliferation acceleration and it has more advanced risk of genetic disorder.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Diploid</th>
<th>Low SPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ovarium</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Head</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lungs</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Colorectal</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stomach</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Melanoma</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Brain</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Limfoma</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Mieloma</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>-</td>
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</tbody>
</table>

* Tannock (5)

CELL KINETIC AS CANCER PROGNOSIS FACTOR

In some of cancer, SPF and polidi status is assumed as correlated to prognosis and therapeutic response. Ito et al reveal that studies of hyperdiploid ALL patients
demonstrate different biological nature to diploid ALL, i.e. hyperdiploid is correlated to the cell death.

Generally, aneuploid tumor has more severe prognosis compared to diploid tumor and high proliferation tumor, which is stated by SPF or LI value. Higher SPF or LI value have more severe prognosis compared to low SPF value tumor. This information may be used as clinical guideline practice. Nevertheless, correlation between proliferative parameter and chemo-therapy response is very complicated. Some researcher reveal that in a group of AML patient, the LI value before therapy is predictive factor for complete remission, while other researcher who have evaluate LI value of adult leukemia reveals that only some of that value that may be used as prediction factor. May be it is caused by measurement method in measuring proliferation level can not give us information about the cell proliferation level and it can not give any information about proliferation level of clonogenic cell (cells that able to form colonization) in tumor, which in fact is the target of given therapy. As illustration, we demonstrate data of ploidi status and SPF profil of various kind of cancer that has been collected at Dharmais Cancer Hospital. Ploidi status and SPF of 195 tissues of various kind tumor have been evaluated and the result may be seen on table 3. We cannot monitor all kinds of cancer course, but we found that aneuploid rectal cancer and NPC plus high SPF have shown good response to radiation. But in nest monitoring, we found that even they show good therapeutic response, 50% of tumor with intermediate SPF and high SPF and 43% of aneuploid tumor are recurrent in 7 month up to 2 years period. From laryngeal cancer, we found that there is significant correlation between ploidi status with metastasis to lymph node, i.e. the risk of metastasis is 1.7 x greater than aneuploid tumor compared to diploid. Significant correlation is also found between SPF and stadium, i.e. the risk of high stadium is 2.5 x greater on high grade SPF tumor compared to low grade SPF tumor. Survival analysis also found that SPF is a better prognostic factor compared to stadium or histological grade. Nevertheless, we still need more data to have some conclusion, it might be that evaluation of proliferation and ploidi status on various kind of tumors may be used to support prognosis determination and as prediction factor of therapeutic response and survival.

REFERENCES


