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**Development of cancer-initiating cells and immortalized cells with genomic instability**

Yoshioka K *et al****.*** CIC development and genomic instability

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**Abstract**

Cancers that develop after middle age usually exhibit genomic instability and multiple mutations. This is in direct contrast to pediatric tumors that usually develop as a result of specific chromosomal translocations and epigenetic aberrations. The development of genomic instability is associated with mutations that contribute to cellular immortalization and transformation. Cancer occurs when cancer-initiating cells (CICs), also called cancer stem cells, develop as a result of these mutations. In this paper, we explore how CICs develop as a result of genomic instability, including looking at which cancer suppression mechanisms are abrogated. A recent *in vitro* study revealed the existence of a CIC induction pathway in differentiating stem cells. Under aberrant differentiation conditions, cells become senescent and develop genomic instabilities that lead to the development of CICs. The resulting CICs contain a mutation in the alternative reading frame of *CDKN2A* (ARF)/p53 module, i.e., in either ARF or p53. We summarize recently established knowledge of CIC development and cellular immortality, explore the role of the ARF/p53 module in protecting cells from transformation, and describe a risk factor for genomic destabilization that increases during the process of normal cell growth and differentiation and is associated with the downregulation of histone H2AX to levels representative of growth arrest in normal cells.

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**Key words:** ARF/p53 module; Cancer stem cells; Cancer-initiating cells; Differentiation; Genomic instability; H2AX

**Core tip:** Cancer usually develops in conjunction with genomic instability and multiple genetic mutations. Only a small number of cells, called cancer-initiating cells (CICs), are the progenitors of cancerous tissue; but how genomic instability and genetic mutations prompt CICs to develop is still unclear. Recent investigations have uncovered the existence of a pathway that could be responsible. This review explores how that pathway might induce the development of CICs, the tumor suppression mechanisms that must be abrogated in order for malignancies to occur, and the role of the ARF/p53 module in protecting normal cells from oncologic transformation.

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**INTRODUCTION**

Somatic stem cells are responsible for the development and homeostasis of organs and other bodily tissues. Cancer-initiating cells (CICs), or cancer stem cells, are responsible for the development[1,2], metastasis, and drug resistance[3-6] of tumors. Although CIC characteristics are increasingly being well defined, how CICs develop remains unclear.

Unlike pediatric tumors, which usually develop as a result of very specific chromosomal translocations[7,8] and epigenetic aberrations[9,10], cancers that become more common around the age of 40 years exhibit extreme chromosomal instability (CIN) or microsatellite instability (MSI)[11-14]. MSI occurs in cells that do not have adequate mismatch repair systems[15-17], and CIN occurs in the presence of other types of repair deficiencies. The hereditary versions of myelodysplastic syndrome[18], breast and ovarian cancers[19-22], and skin cancers[23-25] are examples of CIN. CIN can even occur in normal senescent cells[26,27].

MSI and CIN usually do not occur together and are considered mutually exclusive. For example, 15% of colorectal cancers exhibit MSI, but most of the remainder exhibit CIN. There are also tumors that do not exhibit either MSI or CIN and result from DNA polymerase ε mutations, which interfere with proofreading functions and produce hypermutations[28,29]. Massive genomic rearrangements occur in a wide variety of cancers[30-32], and the unstable structures that result vary widely[33,34]. Since only a very small number of CICs are necessary to produce a malignancy, what causes CICs to develop in the first place is an important question[35,36].

In malignant cells, mutations in either alternative reading frame of *CDKN2A* (ARF) or p53 are common. The ARF/p53 module plays a major role in keeping normal cells from transforming into malignancies, so these mutations that interfere with ARF/p53 functioning show us how the barrier reactions performed by the ARF/p53 module normally work and the way genomic instability promotes the development of CICs.

**EFFECTS OF GENOMIC INSTABILITY**

Like cancer cells that develop because of genomic instability *in vivo*[37-40], cells cultured *in vitro* can also be transformed and/or immortalized in association with either CIN- or MSI-type genomic instabilities[26] and mutations in the ARF/p53 module[41]. Following serial proliferation, normal mouse embryonic fibroblast cells (MEFs) stop reproducing after a growth arrest command issued by the ARF/p53 module[41]. This command prevents cells from immortalizing[27,42]; but immortality can develop if the genome is destabilized[43], clearly demonstrating that genomic instability is a triggering event in cellular immortalization.

Genomic instability probably contributes to the induction of mutations in the ARF/p53 module[27,42]. In MEFs, immortalization can result if genomic instability is induced and/or mutations occur in either ARF or p53 (Figure 1), but is blocked in cells with stable genomes that are under the continuous regulation of the ARF/p53 module[27]. Healthy ARF/p53 regulation is essential for the prevention of cellular transformation and immortalization, but which functions of ARF and p53 are responsible for tumor suppression is controversial.

It was generally believed that p53 suppresses tumors by inducing apoptosis and senescence[44-47], but recent studies have cast doubt on this hypothesis. For example, in multiple transgenic mouse models in which p53 cannot induce cell-cycle arrest and apoptosis after DNA damage, the number of malignancies that form as a result is not significantly higher than the rate of malignancies in normal mice[48-50]. These observations raise questions regarding the exact role of p53 in tumor suppression. One hypothesis is that p53 regulates metabolism by inhibiting glycolysis[51-53] and/or activating the mammalian target of rapamycin pathway[52,54,55].

**EFFECT OF MUTATIONS IN THE ARF/P53 MODULE**

Because ARF and p53 are mutated in cancer cells in a mutually exclusive manner, it is likely that the p53 functions that are essential for cancer suppression are expressed under the control of ARF[56]. Intriguingly, transgenic mice with an extra copy of *Arf* and *p53* (super-*Arf/p53* mice) exhibit both reduced rates of cancer and slower overall aging[41]. Furthermore, MEFs derived from these mice rarely immortalize spontaneously. Therefore, it is likely that the roles of ARF and p53 in cancer suppression are involved in the maintenance of homeostasis *in vivo* and are primarily regulated at the level of individual cells. This cellular-level protection against transformation is generally associated with growth arrest and low levels of the histone H2A variant H2AX[27,57], which is required for active cell growth[58].

The downregulation of H2AX is governed by the ARF/p53 module[27,42]. Growth-arrested cells in the liver, spleen, and other organs of healthy adult mice generally exhibit downregulated levels of H2AX, whereas immortalized/transformed cells, which have mutations in the ARF/p53 module, have normal H2AX levels and exhibit active growth[27,57].

As described above, cellular transformation is suppressed primarily by the induction of growth arrest and the downregulation of H2AX under the control of the ARF/p53 module. It is promoted by genomic instability and the mutations it produces. Unfortunately, when H2AX is downregulated, the mechanisms that repair lesions fail and this is a risk factor for the genomic instability and tumorigenesis seen in many hereditary cancers[[15-25](file:///E:\宋秀霞\新期刊\修回稿\12725\12725-Review.docx#_ENREF_15)]. Despite the aforementioned protective effect of H2AX downregulation, the risk of sporadic cancer development is probably due, in large part, to a reduction in H2AX levels and the associated repair deficiencies[27]. In fact, cells without H2AX exhibit faulty homologous recombination and non-homologous end-joining during DNA repair[59,60], which results in elevated genomic instability[58].

**CIC DEVELOPMENT IS DISTINCT FROM IMMORTALIZATION**

Although immortalized MEFs develop in association with genomic instability and mutations in the ARF/p53 module[26,41], the resultant cells do not exhibit robust tumor-forming ability unless they are pre-transformed by oncogenes[61,62]. This observation illustrates the difference between immortalized MEFs and CICs. MEFs are differentiated cells and do not exhibit stem cell characteristics, but CICs act like stem cells in cancer-tissue development. In fact, CICs share a number of common characteristics with normal stem cells, including embryonic stem (ES) cells and induced pluripotent stem cells, but do not resemble immortalized MEFs with their high dependence on normal glycolysis[63,64], sphere-forming ability, and expressed stem cell marker genes[65-67]. Cancer tissues generally exhibit the “Warburg Effect” seen in normal stem cells that produce elevated glycolysis.

***Transformation of normal stem cells into CICs after genomic destabilization***

To truly understand cancer, we must discover how genomic instability promotes the development of CICs. CICs may develop via multiple pathways. A recent study using normal murine ES cells as a model showed that one of these possible pathways might be created when normal stem cells are developing under unstable genomic conditions. Under aberrant differentiation conditions, ES cells with the ability to form normal mice develop high levels of DNA lesions. These lesions induce cellular senescence and genomic instability, ultimately leading to renewed growth in cells that harbor mutations in the ARF/p53 module[68]. These cells exhibit a number of stem cell characteristics, including sphere formation and the expression of undifferentiated marker genes such as *Nanog*, *Klf4*, *Oct3/4*, and *Sox2*, even in a growth factor–poor medium containing 10% newborn bovine serum with no leukemia inhibitory factor. Furthermore, these cells possess tumor-forming ability and express c-Myc and CIC markers such as CD133, CD33, and CD34.

The fact that oncogenesis can be triggered by niche disruption supports this theory. For example, both leukemia and myelodysplasia develop after dysregulation of the stem cell niche[69], and cancers often develop from stem cells that are injected at heterotropic sites[70]. Stem cells growing in such an aberrant environment do not get what they need to maintain themselves properly and often transform into CICs[70]. Another example is embryonal carcinomas that can develop from cells transplanted from the inside of blastocysts and from primordial germ cells derailed from the migration track[71,72]. These *in vivo* and *in vitro* findings suggest that the genomic instability that leads to the development of CICs can be triggered by aberrations in the environment when stem cells are differentiating.

Because cancer development is initiated by CICs, these cells are considered promising targets for chemotherapy aimed at either killing them directly or getting them to differentiate[73-75]. However, this strategy is not always successful, partly because CICs are so adaptable and plastic[76,77]. In one study in which CICs developed from ES cells, transformation was coupled with the acquisition of plasticity. ES cells start to differentiate in response to leukemia inhibitory factor withdrawal and become senescent when differentiation conditions are aberrant; but the CICs that result re-acquire stem cell characteristics as they develop genomic instability, even under conditions in which stem cells ordinarily do not thrive and cannot be cultivated[68]. CICs that re-acquire stemness under these conditions maintain stem cell characteristics with great persistence, and it is difficult to get them to differentiate completely.

**RELEVANCE OF *IN VITRO* MODELS TO CANCER DEVELOPMENT**

Unlike *in vitro* malignancy transformation, *in vivo* cancer development has multiple steps that include abrogating organic regulation, which results in the development of benign tumors such as adenomatous polyposis coli mutations in the colon[78,79], and losing the ARF/p53 module that leads to uncontrolled growth and the development of malignancies[[80](file:///E:\宋秀霞\新期刊\修回稿\12725\12725-Review.docx#_ENREF_84),[81](file:///E:\宋秀霞\新期刊\修回稿\12725\12725-Review.docx#_ENREF_85)]. Each organ in a complex animal is regulated by its own specific mechanisms that keep the organ in homeostasis, which can malfunction and allow tissues to become precancerous or malignant when the genome becomes unstable or the mutations affect the ARF/p53 module[40,56].

**CONCLUSION**

Genomic instability contributes to the development of CICs by directly transforming somatic stem cells, reprogramming differentiated cancer cells, and a number of other mechanisms. Several lines of evidence suggest that one of these pathways is triggered by the disruption of the niche environment (Figure 2). Maintenance of the niche environment is essential for somatic stem cell maintenance and differentiation and homeostasis (Figure 2A). The stem cell niche can be damaged by exogenous stresses that cause irregular stem cell differentiation, the accumulation of DNA damage, and the induction of senescence (Figure 2B). DNA damage often triggers genomic destabilization, which can promote the development of precancerous (Figure 2C) and cancerous (Figure 2D) lesions through the maintenance of small pockets of pluripotent stem cells that eventually become CICs.

If stem cells start to differentiate under unfavorable differentiation conditions, they become senescent, but can start developing again and reacquire stem cell characteristics when conditions change. Stemness recovered under these circumstances is associated with robust plasticity and the ability of these cells to self-renew, even under conditions in which normal stem cells do not thrive. Unfortunately, such cells do not differentiate completely and remain permanently in less developed states that often lead to malignancies.

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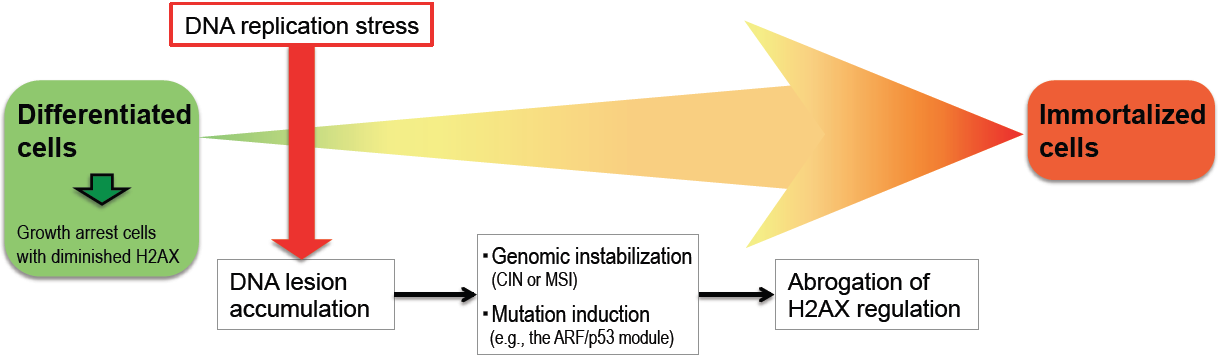
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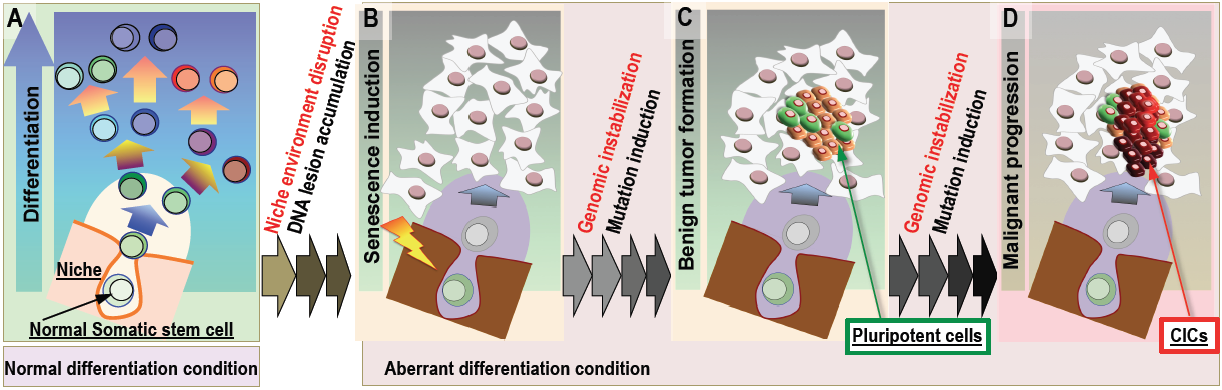
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**Figure 1 Model of cellular immortalization in association with genomic instability.** As illustrated in mouse embryonic fibroblasts, immortality is induced by genomic instability and mutation of either *Arf* or *p53*. Growth arrest and the downregulation of H2AX protect mouse embryonic fibroblasts against immortalization. Because H2AX downregulation is dependent on the ARF/p53 module, cells with mutations in this module recover H2AX expression and growth activity. CIN: Chromosomal instability; MSI: Microsatellite instability.



**Figure 2 Model of cancer-initiating cell development.** A: In normal tissues, stem cells are maintained in a specific niche and the niche environment is important both for stem cell maintenance and organ homeostasis; B: Niche disruption deranges the differentiation environment of stem cells, leading to the accumulation of DNA lesions and the induction of cellular senescence. Such DNA lesions trigger genomic destabilization, leading to the development of benign tumors that contain a small number of C: Pluripotent cells that eventually develop into; D: Cancer-initiating cells (CICs) that cause malignancies.