A Possible Role of Stress-Induced Premature Senescence, SIPS, as a Producer of the Stress-Resistant Microenvironment

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The microenvironment is consisted both of soluble factors involving growth factors and of insoluble factors. Stroma cells contribute to form the microenvironment through a secretion of these factors. Fibroblast, which is known as stroma cells, also secretes various soluble/ insoluble factors, but the secretion level is significantly up-regulated when they reach to a finite replicative lifespan. Recent accumulating studies not only in vitro but also in vivo provide us that secreted proteins from senescent cells promote pro-survival pathway in bystander cells, especially tumor cells rather than normal cells. Since various stresses including ionizing radiation prematurely induces cellular senescent stage, called Stress-Induced Premature Senescence (SIPS), there is the possibility that the secretion pathway in cells undergoing SIPS is also activated. Here, we propose that pro-survival factor is secreted from SIPS cells to provide the stress-resistant microenvironment.

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SIPS-induction by ionizing radiation

SIPS is defined as stress-induced premature senescence. Toussaint and colleges originally found that sub-toxic hydrogen peroxide stress prematurely introduces senescent phenotype, which is similar to the phenomenon as replicative senescence, into normal human fibroblast.[1] And further studies indicated that various cellular stresses, such as ionizing radiation, ultraviolet radiation, and chemotherapeutic agents also caused premature senescence.[2-9] Not only normal human fibroblast but also many types of cells including melanocytes, endothelial cells, and retinal pigment epithelial cells undergo SIPS by these treatments, and sub-toxic doses of these stresses are required to efficiently induce SIPS. Interestingly, SIPS, instead of apoptosis, is the major response in these types of cells *in vitro*, as well as *in vivo*.[2,10] Nowadays, "SIPS" becomes the most common name to describe the induction of senescent phenotype by any kinds of stress, however, SIPS induced by especially ionizing radiation will be described in this review.

Cells undergoing SIPS share many cellular and molecular features with cells undergoing replicative senescence. On the basis of the telomere hypothesis, replicative senescence is programmed when dysfunctional telomere is appeared. Following the appearance of dysfunctional telomeres, the ataxia telangiectasia mutated (ATM)-p53 pathway is activated and greatly contributes to irreversible growth arrest. Induced p16 levels also contribute to maintain cells in an arrested state.[11] Eventually, two different pathways, the ATM-p53-p21 and p16, appear to be regulated by a dysfunctional telomeric signal and a non-telomeric signal, respectively that redundantly maintain irreversible growth arrest in senescent cells. Senescent cells show specific features to be different from proliferative cells on their morphology and gene expression pattern.[12] A typical senescence-specific morphology is described as 'flattened and enlarged' shape. Age-related gene expression changes have been reported, and some of these are used as "markers" of senescence. Senescence-associated ß-galactosidase (SA-ß-gal) activity is commonly used as a senescent marker. Since this enzyme works under acidic pH conditions in a senescence-specific manner, senescent cells can be recognized as positive cell by this assay.[13]

SIPS is not triggered by dysfunctional telomeres and just response to an exposure of ionizing radiation. A common feature is that DNA-damage checkpoint machineries have been permanently activated with same pathway shown in cells undergoing replicative senescence. Irreparable damages at telomere-nonspecific sites serve as a signal to induce and maintain SIPS. Cells undergoing SIPS show senescence-like growth arrest (SLGA), which represents irreversible growth arrest with features of senescence-specific cell morphology and gene

expression.[14] Through p53 stabilization and transactivation, as well as the p16 pathways appear to maintain SLGA. DNA double-strand breaks (DSBs) created by ionizing radiation result in an activation of ATM-p53-p21 pathway, in turn, p21-mediated G_1 growth arrest is activated. p53 accumulation and phosphorylation at Ser15 occurs within 2-4 hr after irradiation.[15] Normally, in cells that recover from this initial insult p53 responses wane within 6 hr post-irradiation. However in SIPS cells, these responses have been continuously observed at least for 10 days.[2] p16 shows delayed expression compared to the induced p53 pathway. p16 induction is observed 5 days after treatment.[2] SA-ß-gal staining appears from 3 days after exposure to 4 Gy of X-rays, and nearly all the remaining irradiated cells show SA-ß-gal-positive staining 5 days after treatment.[2] These responses are observed in a dose-dependent manner.

These observations highlight the fact that ionizing radiation-induced DNA damage persists in cells undergoing SIPS. Ionizing radiation-induced foci (IRIF)-formation peaks within 30 min in irradiated cells, followed by their decay in two steps: (i) the first decay rate of IRIF disappearance is rapid and within 4 hr after ionizing radiation exposure most foci disappear; (ii) However, during the second decay rate a much slower reduction in the kinetics is apparent.[16] This two-step decay rate is directly related to the kinetics of DSB repair.[17] Many IRIF disappear within 1 day after irradiation, however, a few IRIF are still observed in each irradiated cell after high dose ionizing radiation exposures, and these foci persist for at least 5 days post-treatment.[16] p53 immunostaining data show that p53 accumulates in the nuclei of cells with substantial IRIF. Interestingly, p53 also aggregates around the site of IRIF. The phosphorylated form of p53 at Ser15 is, particularly, detected at IRIF, suggesting that p53 is directly activated by ionizing radiation-induced damage during SIPS-induction.[16]

Senescence associated secretory phenotype

Recent accumulating data reveled one more feature shown in cells undergoing replicative senescence, that is senescence associated secretory phenotype (SASP). Basically, proliferating fibroblasts secrete many kinds of proteins such as growth factors, cytokines, fibronectin and so on. When fibroblasts undergo replicative senescence, senescent cells show specific morphology and biochemical marker, and loose the capacity to proliferate, yet these cells remain metabolically alive. Krtolica et. al. suggested the possibility that the secretion process of growth factor is up-regulated in senescent cells.[18] Although co-culture of normal epithelial cells with proliferating fibroblasts promotes cell growth of epithelial cells compared with that of epithelial cell alone, further promotion of cell growth in epithelial cells was observed when epithelial cells were co-cultured with replicative senescent cells. Furthermore, this promotion on cell growth was more significant in tumor cells rather than normal cells, suggesting the secretion of growth factors up-regulated from replicative senescent cells markedly support tumor growth. Same results were obtained by co-culture with cells undergoing SIPS. Interestingly, the downstream effects caused by secreted factors from senescent cells

depends on the recipient cell type, and also secretion pattern depends on stromal cell types.[19] In case of SIPS, various senescent cancer cells may secreted tumor-growth promoting proteins (i.e., TGFα, angiogenic factor cyr61, and the anti-apoptotic factors, Galectin-3 and Prosaposin), as well as tumor-growth suppressing factors (i.e., IGFBP-3, 4, and 6 and MIC1).[20-23] Depending on the localization of the tumor, accumulation of senescent cells may have profound affects on the survival responses of cancer cells.

Possible function of the microenvironment for radiosensitivity

The microenvironment is consisted of soluble factors such as growth factors, cytokines, and chemokines, and of insoluble factors which form extracellular matrix. Stroma cells contribute to form the microenvironment through a secretion of these factors. Fibroblast, which is known as stroma cells, also secretes various soluble/ insoluble factors, but the secretion level is significantly up-regulated from cells undergoing replicative senescence or SIPS. Recent accumulating studies not only in vitro but also in vivo provide us that secreted proteins promote pro-survival pathway in bystander cells, especially tumor cells rather than normal cells. There are some perspective mechanisms that the microenvironment formed by cells undergoing SIPS promotes stress-resistancy to bystander cells. One is that growth factors activate pro-survival pathway in bystander cells. It is reported that various growth factors including IGF-1 and VEGF are up-regulated and are secreted after exposure to ionizing radiation.[24,25] Yu et. al., reported that IGF-1 receptor which activates IGF-1 receptor-related signaling pathway by binding of IGF-1 ligand, regulates radiosensitivity.[26] Mutational analysis on IGF-1 receptor revealed that C-terminal of this receptor contributed to abrogate radioresistance, and this effect might be independent of PI3K/ ERK signaling. And other possibility to increase radiosensitivity is that signaling pathway activated by growth factors promotes the expression of pro-survival pathway. On this point of view, it is suspected that high expression level of secretory clusterin (sCLU) gives radioresistancy. It has been clinically observed that sCLU was overexpressed in tumor samples derived from brain, lung, breast, and prostate, and tumor cells overexpressed sCLU showed radioresistancy.[27] Once sCLU was knocked down, ionizing radiation introduce cell death into tumor cells knocked down sCLU, suggesting sCLU modulates radiosensitivity. Criswell et. al., revealed the induction mechanism of sCLU by ionizing radiation which is IGF-1/ IGF-1 receptor-related signaling pathway regulates sCLU induction.[24] Not only the induction of sCLU but also the internalization of sCLU can contribute to give radioresistance. Megalin receptor (or gp330) is especially expressed in mammary gland cells, and sCLU may be endocytotically internalized via this receptor and potentially functions as pro-survival factor.

In this review, we focused on a possible function of SIPS to form stress-resistant microenvironment. Accumulating studies described candidates for this possibility, but it is not clear how secreted factors from SIPS contribute to form radioresistant microenvironment. We

proposed that growth factors can activates pro-survival pathway, but it is also known some growth factor-binding proteins secreted from cells undergoing SIPS. So we should clarify that secretion profile of those factors and figure out the mechanism to form radioresistant microenvironment.

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