Senescence, apoptosis, and stem cell biology: the rationale for an expanded view of intracrine action

Richard N. Re and Julia L. Cook
Ochsner Clinic Foundation, New Orleans, Louisiana

Submitted 4 May 2009; accepted in final form 2 July 2009

Re RN, Cook JL. Senescence, apoptosis, and stem cell biology: the rationale for an expanded view of intracrine action. Am J Physiol Heart Circ Physiol 297: H893–H901, 2009. First published July 10, 2009; doi:10.1152/ajpheart.00414.2009.—Some extracellular-signaling peptides also at times function within the intracellular space. We have termed these peptides intracrines and have argued that intracrine function is associated with a wide variety of peptides/proteins including hormones, growth factors, cytokines, enzymes, and DNA-binding proteins among others. Here we consider the possibility that intracrines participate in the related phenomena of senescence, apoptosis, and stem cell regulation of tissue biology. Based on this analysis, we also suggest that the concept of intracrine action be expanded to include possible regulatory peptide transfer via exosomes/microvesicles and possibly by nanotubes. Moreover, the process of microvesicular and nanotube transfer of peptides and other biologically relevant molecules, which we inclusively term laterality, is explored. These notions have potentially important therapeutic implications, including implications for the therapy of cardiovascular disease.

These notions have potentially important therapeutic implications.

Cellular senescence was first demonstrated by Hayflick in the 1960s through his observation that cultured fibroblasts cease dividing after a fixed number of divisions despite the continued presence of growth factors in the culture medium (31). The cells do not die but enter a state of irreversible growth arrest associated with specific morphological changes. The causes of senescence are complex, but a common feature of this form of senescence appears to be the loss of telomeric DNA with each cell division, such that after a finite number of divisions in the absence of the restorative enzyme telomerase (found in immortal germ cells and neoplastic cells), cell division ceases. If senescence-related growth cessations do not occur in these cells, genomic catastrophe ensues (2, 3, 24). Recent data raise the possibility that telomere shortening per se is not the trigger for senescence, but rather the disruption of the telomere cap and exposure of the 3′ overhang (as occurs with severe telomere shortening) is a trigger (45, 56). It has also been found that cells assume a very similar senescent phenotype if they are subjected to DNA damage (ultraviolet light) or oncogene activation. After such insults, cells are often found to become apoptotic on the one hand or senescent on the other, apparently dependent on the degree of DNA damage present. The pleiotropic protein p53, as well as the tumor suppressors p16 and retinoblastoma protein, is involved in the regulation of this process, which is unrelated to telomeric shortening, but they also play a role in the replicative senescence that is related to shortening. These processes are complex and incompletely understood and are described in detail elsewhere (2, 3, 10, 18, 19, 24, 31, 36, 67). It is clear, however, that in abnormal cells, p53 can induce transient cell-cycle arrest, permanent arrest (senescence), or apoptosis, depending on the context (18, 67). It is also widely believed that senescence evolved to protect organisms from neoplasia by arresting the growth of cells displaying genomic damage. This mechanism would prevent the neoplastic transformation of long-lived pluripotent tissue progenitor cells (tissue stem cells) (22, 49). Similarly, apoptosis can intervene in some instances to protect the organism from cancer. Somewhat surprisingly, however, the factors secreted by senescent cells, like those secreted by some stem cells, can also alter the microenvironment to enhance the growth of neighboring tumor cells, indicating that this protective mechanism can act as a double-edged sword (1, 18, 19, 22, 49).

Recently, it has been reported that in both replicative and cell damage-driven senescence, cells assume a novel phenotype, the so-called senescence-associated secretory phenotype (SASP), which is characterized by the secretion of a wide variety of factors, including peptide hormones, as well as the release of small membrane-bound bits of cytoplasm, so-called...
Perspectives

Table 1. Intracrines

<table>
<thead>
<tr>
<th>Hormones, Cytokines</th>
<th>Growth Factors</th>
<th>DNA-Binding Proteins</th>
<th>Enzymes</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>FGF-1, -2, -3, -10</td>
<td>Homeoproteins</td>
<td>Phosphoglucoisomerase/</td>
<td>Lactoferrin</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>Midkine</td>
<td>Amphoterin (HMGBl)</td>
<td>Renin/prorenin (aspartyl</td>
<td>Endogenous opioids (dynorphin)</td>
</tr>
<tr>
<td>Prolactin</td>
<td>VEGF</td>
<td>IL-33</td>
<td>protease)</td>
<td></td>
</tr>
<tr>
<td>Interferon-β, -γ</td>
<td>NGF</td>
<td></td>
<td>Granzyme A, B</td>
<td></td>
</tr>
<tr>
<td>Interleukins</td>
<td>PDGF</td>
<td></td>
<td>Phospholipase A2-1</td>
<td></td>
</tr>
<tr>
<td>Oxytocin</td>
<td>Pleiotrophin</td>
<td></td>
<td>Urokinase</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>Proenkephalin</td>
<td></td>
<td>Lysyl-RNA synthetase</td>
<td></td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Pigmented epithelium-derived factor (a serpin)</td>
<td></td>
<td>Thiolodoxin</td>
<td></td>
</tr>
<tr>
<td>Somatostatin</td>
<td>Maspin (a serpin)</td>
<td></td>
<td>Tyrosyl-RNA synthetase</td>
<td></td>
</tr>
<tr>
<td>TRH</td>
<td>Schwannom derived growth factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LHRH</td>
<td>Leukemia-inhibiting factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>Macrophage colony-stimulating factor-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP</td>
<td>Hepatopoietin</td>
<td></td>
<td>Angiogenin</td>
<td></td>
</tr>
<tr>
<td>Gonadotropin</td>
<td>TGF-α</td>
<td></td>
<td>Acetylcholinesterase readthrough</td>
<td></td>
</tr>
<tr>
<td>Chorionic gonadotropin</td>
<td>Hepatopoietin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin (1-7)</td>
<td>Herengulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelin</td>
<td>TGF-β</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythropoietin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Intracrines are categorized by their best-studied mode of action; they are independently listed in each column and are not associated across rows. PTHrP, parathyroid hormone-related protein; TRH, thyrotropin-releasing hormone; LHRH, luteinizing hormone-releasing hormone; VIP, vasoactive intestinal peptide; ANP, atrial natriuretic peptide; HMGBl, high-mobility group box 1; PD-ECGF, platelet-derived endothelial cell growth factor; Tat, human immunodeficiency virus transactivator; IGFBP, insulin-like growth factor-binding protein. See Refs. 11, 20, 25, 30, 37, 41, 47, 48, 50, 58–66, 69, 71, 72, 74, 75, 83.

It has also been shown that embryonic stem cells (ESCs) and some mesenchymal stem cells (MSCs) can regulate the apoptosis of injured cells on the one hand and tumor aggressivity on the other by altering the local microenvironment (22, 44, 76, 80). The argument has been made that cancer is perpetuated by stem-like cells (cancer stem cells), which bear some resemblance to tissue stem cells but lack the regulatory systems of the normal cells (66). The colocalization of tumor and stem cells of various sorts has been suggested as a means of providing missing stem cell regulatory factors to tumor cells to mitigate their proliferation, and there is evidence to support this idea (49). In fact, it has been suggested that most cancers derive from transformed tissue progenitor cells. If so, the similarities between stem cells and the hypothesized cancer stem cells become more explicable and the possible reconstitution of normal regulatory pathways by stem cells in cancer cells seems more feasible (39, 49). In this regard, we have argued that stem cell biology is in part regulated by intracrine functionality and have also suggested a role for intracrine action in the maintenance of tumor cell populations by cancer stem cells (66).

These collective findings are relevant in the present context because some of the peptide factors secreted by SASP-senescent cells are, in fact, intracrines [IL-1α, IL-6, maspin, VEGF, insulin-like growth factor-binding protein (IGFBP)-3], and others may be as well [IL-8, growth-related oncogene (GRO)α, plasminogen inhibitor activator-1, and IGFBP-7] (2, 10, 18, 23, 24, 66, 67, 80). Also, any intracellular peptide regulatory molecule that traffics between cell interiors via exosomes is arguably an intracrine as well. Intracrine factors are associated with tissue and cellular differentiation, and arguably, senescence is a form of differentiation. Similarly, there is good evidence for the participation of intracrines in stem cell biology, and it is reasonable to ask whether intracrines participate in the cancer suppression actions of some tissue progenitor cells. The issue then becomes, Is intracrine functionality important in the development of the senescent phenotype (as it appears to be in some forms of differentiation) or is it simply...
a curiosity that some of the factors secreted by SASP cells may in other contexts act in the intracellular space? The answer to this question could have important biological and therapeutic implications. Similar questions can be raised about the mechanisms by which stem cells, be they embryonic or tissue, modulate the activity of neighboring cancer cells or damaged tissue cells. To approach this question, several specific examples of intracrine participation in the SASP, apoptosis, and ESC-induced cancer suppression will be considered. Although in most cases no definitive answers can yet be given to the role of intracrine functionality in these processes, a considerable amount of suggestive evidence has accumulated regarding this question and the data available have implications for the pathogenesis and therapy of cancer as well as atherosclerotic disease. The release of exosomes/microvesicles by senescent cells and some stem cells also suggests a wider interpretation of intracrine function.

p53 AND SASP

The SASP has been associated with the secretion of a wide variety of factors including extracellular proteases, matrix components, cytokines, and chemokines. Some of these factors such as VEGF are proangiogenic or supportive of tumor cell growth, whereas others reinforce the senescent phenotype. This highlights the tumor-suppression and tumor-enhancement dual role of the SASP. The prevailing opinion is that the SASP reinforces the senescent phenotype in senescent cells and possibly induces the phenotype in nearby cells. Prominent factors secreted in SASP are the intracrines IL-1α, IL-6, and the CXC chemokine receptor (CXCR)1/CXCR2 ligands IL-8, and GRO-α, as well as IGFBP-7 (2, 10, 18, 23, 24, 67, 80). It is interesting to note that in tumor cells, IL-6 and, apparently, IL-8 form positive feedback loops as is common among intracrines to support tumor growth (6, 50). IL-6 is known to use intracellular-positive feedback loops in maintaining the growth of renal carcinoma cells, and therefore IL-6 is an intracrine (6). This suggests the possibility that IL-8 and its fellow CXCR2 ligand GRO-α could be intracrines. IGFBP-3, which is also secreted by some senescent cells, and IGFBP-5 are intracrines, raising the possibility that IGFBP-7 is as well (see below) (33, 37, 63–66). In addressing these questions, it can be noted that IL-8 and GRO-α can be found along with their receptor diffusely in the cytoplasm of cells. Moreover, the related receptor CXCR4 has been demonstrated in cell nuclei (77). A recent report provides immunohistochemical evidence to tentatively support the presence of the CXCR2 receptor in the nucleus (78). Thus there is circumstantial evidence that IL-8 and probably the related ligand GRO-α can function as intracrines. Indeed, as is common for intracrines, the CXCR2 ligands IL-1, IL-6, IL-8, and GRO appear to participate in positive feedback loops that maintain the senescent state and parallel the positive loops that characterize intracrine differentiation; however, it is unclear whether these factors actually function in an intracrine (i.e., intracellular) mode (10). There is also evidence indicating that the known intracrine IGFBP-3 is upregulated in senescent fibroblasts and in some senescent endothelial cells. Moreover, the overexpression of IGFBP-3 leads to endothelial cell senescence. Also of note, in colon carcinoma cells, IGFBP-3 can be shown to be upregulated by p53 (80).

In addition to regulating the secretion of the various factors discussed above, p53 also regulates the release of so-called “exosomes,” 50–90-nm-diameter closed bits of cytoplasm carrying cytoplasmic materials including growth-regulating factors and RNA (exosomes are generally considered to be derived from endosomes, endoplasmic reticulum, or related structures, whereas a microvesicle is usually used as a more general term and includes vesicles containing cell surface membrane components) (4, 15, 35, 42). In some cases these exosomes can fuse with the outer cell membrane of target cells, thereby releasing their contents into those cells. A variety of cells including dendritic cells, reticulocytes, B cells, T cells, epithelial cells, and tumor cells have been shown to release exosomes. In cells undergoing stress of various sorts including those leading to senescence, p53 acts as a transcription factor and upregulates a wide variety of secreted factors including the intracrine IGFBP-3, plasminogen inhibitor activator-1, and thrombospondin (24, 67, 80). In addition, p53 upregulates tumor suppressor-activated pathway-6 transcript (TASP6), an intracellular factor that stimulates exosome release into the extracellular medium (44, 80). Among the factors contained in these exosomes is the antiangiogenic intracrine maspin and the intracrine translationally controlled tumor protein (11, 44, 80). Translationally controlled tumor protein, also known as mortalin, is a secreted protein that stimulates the release of histamine from eosinophiles and other leukocytes; in the cell it is a tubulin-binding protein and mitotic spindle protein that affects cell-cycle regulation. It also acts as a transcription regulator controlling Oct-4 and Nanog expression. It is therefore an intracrine that can traffic via exosomes. It is not known whether it gains access to target cell interiors after exosome transport or whether instead it acts at the target cell extracellular membrane. Exosomes also contain both mRNA, which is translationally competent, as well as microRNAs (miRNAs); this RNA has been called “exosomal shuttle RNA” (15, 35, 42, 57, 76, 80, 81). The potential physiological importance of exosome release can be seen when considering the biology of EGFR-bearing tumor cells. Specifically, exosome-like microvesicles can be released by EGFR-bearing tumor cells, whereupon the vesicles are incorporated by neighboring endothelial cells with the resultant activation of the MAPK and Akt pathways and the autocrine production of VEGF; thus microvesicle release by tumor cells influences the microenvironment and regulates tumor angiogenesis. Indeed, microvesicle transfer between EGFR-bearing glioma cells can accelerate the growth of tumor cells that take up the vesicles (7). Thus the effects of exosomal release in senescence could also be wide ranging and could involve both intracrine and nonintracrine action.

Collectively, these findings indicate that p53, in addition to its role in initiating alternatively apoptosis or senescence, is an important regulator of both secreted and exosomal cellular regulators. This extends the concept of intracrine to exosomes and strongly suggests that intracrines participate in the SASP and the maintenance of senescence. Moreover, these findings suggest that intracrines could be useful in the suppression of pathological proliferation through the establishment of the senescent state. This idea gains support from recent observations of the biology of the aforementioned IGFBP-7/IGFBP-rP1. IGFBP-rP1...
also binds insulin, leading to insulin resistance. Recent evidence indicated that this protein induces senescent growth arrest in colorectal cancer cells. Also, cancer cells engineered to overproduce this protein develop a senescent phenotype. IGFBP-rP1 is also synthesized by colorectal carcinoma cells and is associated with a less aggressive phenotype. In some cell lines IGFBP-rP1 induces apoptosis, again a cancer-suppressing effect (22, 41, 47, 75). Based on the analysis above, one could, by analogy, suspect that this protein, like some other members of the IGF-binding protein superfamily, is an intracrine. In fact, a review of the literature shows this to be the case: IGFBP-rP1 is found in the nuclei of prostate carcinoma cells where it appears to reduce the proliferative capacity of the cells (75). A specific intranuclear-binding partner for IGFBP-rP1, neuroendocrine differentiation factor/25.1, has been identified, and it appears that the interaction between these two proteins promotes the differentiation of prostate carcinoma cells into neuroendocrine-like cells (75). Thus there is good evidence that IGFBP-rP1 is an intracrine which is produced by tumor cells, resulting in the suppression of the neoplastic phenotype and which when exogenously administered induces a clear senescent phenotype in those malignant cells (47, 75).

\[\text{Stem Cells and Cancer Suppression}\]

ESCs employ a series of factors to maintain their stemness (1, 53, 55, 66). Among these, for example, is Nodal, a member of the TGF-β superfamily. Nodal prevents ESC differentiation. Because of the similarities between ESCs and tissue stem cells on the one hand and cancer-producing cells, possibly the hypothesized tumor stem cells, on the other, it was asked whether the factors elaborated by stem cells could modify the behavior of tumor-producing cells (1, 32, 51–55, 57). It was found that the exposure of melanoma cells to a zebrafish embryonic environment results in the cells reprogramming to a less malignant phenotype. Of note, Nodal expression is upregulated in aggressive melanoma and breast cancer cells. Nodal participates in a positive feedback loop, upregulating its own synthesis and also that of its inhibitor Lefty. However, Lefty is expressed in ESCs but not in melanoma cells. Thus it may be that the salutary effects of embryonic cells on melanoma result from the provision of Lefty to the tumor cells (1, 32, 53–55). At present there is no evidence to support the possibility that Nodal or Lefty functions as intracrines; i.e., there is no evidence that they act in the intracellular space. However, it has also been shown that MSCs can reduce apoptosis in irradiated fibroblasts and in lung epithelial cancer cells by secreting soluble factors. One of these is the calcium-regulating protein stanniocalcin-1 (STC). STC is a secreted protein that is widely expressed. Among its function is a binding to renal proximal tubules to increase phosphate transport. It is also found in the mitochondria and the nucleus and therefore is an intracrine. In irradiated fibroblasts, intracellular STC is decreased, but in cells whose apoptosis is blocked by MSC-conditioned medium, intracellular STC, including STC closely associated with focal-adhesion sites, is increased as a result of STC uptake. Of note, the antibody to STC partially blocked the effect of MSC-conditioned media on irradiated fibroblasts and the knockdown of STC in MSCs by small interfering RNA prevented the beneficial effects of MSCs on fibroblast apoptosis. Interestingly, the addition of recombinant STC to irradiated fibroblasts did not have an antiapoptotic effect. This either means that STC is necessary but not sufficient to reduce apoptosis or that recombinant STC is not taken up by target cells, possibly because of the lack of posttranslational modification (16, 20, 25, 30, 48, 69). In any case, STC appears to function in an intracrine fashion in the antiapoptotic action of MSCs.

It has also been shown that ESCs have the capacity to release microvesicles containing biologically active molecules (7, 57, 81). These vesicles can be taken up by nearby target cells, leading to a phenotypic change in those cells. Indeed, in one system, ESC microvesicles appear to be enriched in mRNA including mRNA coding for Wnt; these vesicles are also enriched for the Wnt protein itself. When these microvesicles are incorporated into hematopoietic progenitor cells, stem cell transcription factors Oct-4, Nanog, Rex-1, and HoxB4 among others are upregulated. This upregulation could be the result of the induced synthesis in the target cells by Wnt or the transfer of appropriate mRNA—or even the transcription factors themselves—in the microvesicles. In fact, the microvesicles were shown to contain both Oct-4 protein as well as Oct-4 mRNA, which was subsequently translated (57). Oct-4 is a homeodomain transcription factor and, like other homeoproteins, is expected to be capable of atypical secretion and internalization by target cells; i.e., it has the potential to act in an intracrine fashion (43). Oct-4 microvesicular transfer represents yet another intracrine mode of action for this protein; moreover, the upregulation of Oct-4 expression following microvesicular transfer is consistent with the positive-feedback loops that characterize the actions of many intracrines. In addition, ESC microvesicles have been shown to transfer regulatory miRNAs between cells. Similarly, cancer cells can release microvesicles with attendant effects on their local environment. For example, cancer cells can shed microsomes carrying activated epidermal growth factor receptors with their subsequent uptake by endothelial cells. After the incorporation into target cells, the activated EGFR has the effect of upregulating endothelial cell synthesis of VEGF and VEGF receptor, thereby switching the endothelial cells to an angiogenic phenotype (7). Apoptotic cancer cells can also release so-called apoptotic bodies and, in that way, transfer DNA and even chromatin to nearby cells such as fibroblasts; in cells possessing active p53, cell cycle arrest or senescence occurs, but in p53 deficient cells, the transferred DNA persists and changes the phenotype of the host cell toward a more malignant form (15). Collectively, these phenomena potentially extend the concept of intracrine functionality insofar as peptide extracellular-signaling factors are transferred to the interiors of target cells by this mechanism (it must be noted, however, that our working definition of intracrine function requires that the candidate intracrine peptide be found in the intracellular space, unassociated with elements of the peptide secretory or degrading systems such as secretory granules; this is so because the vesicular components of the hormonal secretory and degrading systems are arguably extensions of the extracellular space) (58–66). Similarly, these findings extend the concept of intracrine function insofar as intracellular regulatory proteins such as transcription factors are transferred to the interiors of distant cells, as if after secretion and internalization. To see this latter point, note that the biological result of the secretion and target cell uptake of an intracrine homeoprotein transcription factor is equivalent to
that of the trafficking of a nonintracrine transcription factor to a target cell in an exosome. Moreover, some cells, for example, so-called interstitial Cajal-like cells, can extend long filamentous tubules, nanotubes, in tissues between cells and may transfer intracellular regulators and organelles between cells by this mechanism. This kind of cell has even been reported in the normal human heart. In other systems, the transfer of endosomes through similar nanotubes has been reported, although the trafficking of soluble cytosolic molecules appears to occur less frequently. Also, MSCs have been reported to rescue injured cells by transferring mitochondria via endosomes, nanotubules, and possibly other routes as well (38, 68, 70). The intercellular transfer of protein-regulatory factors via nanotubes, if it occurs, would be reminiscent of the transfer of transcription factors between plant cells via specialized tubules called plasmodesmata, itself a form of intracrine action (58, 60–62, 64). Thus the exosomes of senescent cells, like the intracrices directly secreted by these cells, could represent a form of intracrine functionality by introducing extracellular signaling molecules into the intracellular space of target cells. So too could the microvesicles released by ESCs and cancer cells. Arguably, microvesicle- and nanotube-mediated intracrine functionality could represent a fourth and fifth mode of intracrine action (Fig. 1). Although these findings expand our appreciation of intracrine action in human biology, this is not unexpected, given the prototypical plasmodesmata-mediated intracrine action already established in higher plants.

Therapeutic implications. The above discussion makes clear that senescence is an active process and that factors secreted by senescent cells can either influence the senescence of nearby cells or can serve as a supportive niche for cancer cells. Thus the paracrine actions of senescent cells must be better understood before therapeutic application can be considered. Stem cells of various sorts also appear to alter their environment with important effects on neighboring cells including cancer cells. The idea that ESCs can influence the proliferation or phenotype of transformed cells is under active investigation. Here, however, we will focus on the roles of senescence and apoptosis in the cardiovascular system where the results described above have parallels in the approach to atherosclerosis and myocardial preservation. Apoptosis has been shown to occur in atherosclerosis, involving vascular smooth muscle cells and endothelial cells alike (73). Similarly, a small but growing body of data suggests that senescence, as measured by a decreased telomere length, is associated with hypertension and atherosclerosis in humans (5, 8, 9, 13, 14, 17, 21, 26, 79).

Considerable recent investigation has centered on the possibility that senescence plays a role in cardiovascular disease and atherosclerosis. Just as shortened telomeres are found in chromosomes of persons of advancing age, so too shortened telomeres, measured in circulating leukocytes, have been reported in association with low HDL cholesterol in childhood, with high-circulating concentrations of aldosterone, with increased carotid intimal medial thickening, with carotid plaque in hypertensives, and with a predisposition to coronary artery disease in specific patient populations (5, 8, 9, 13, 14, 17, 21, 26, 27, 68, 70, 73, 79, 82). Although this is not conclusive evidence for a role of senescence in cardiovascular disease, these findings are suggestive. However, one is left to ask exactly how telomeric shortening is related to the progression of cardiovascular disease. Although studies have shown that with aging the percentage of actually senescent cells in tissues increases, these cells nonetheless constitute only a small fraction of the tissue cell population. And, although telomeric damage can upregulate p53, thereby making cells more susceptible to cell-cycle arrest following growth factor deprivation, no pathological effects of telomere shortening other than the triggering of senescence (by either the sensing of a 3’ overhang or of extreme shortening) have been described (3, 13, 36, 45, 56). This then raises the possibility that the SASP could explain functional tissue aging in the presence of relatively few senescent cells; i.e., the factors, including the intracrices, secreted by senescent cells could impair the function of nearby cells without driving them to the canonical senescent phenotype. These factors could also suppress reparative endothelial progenitor cell (EPC) numbers or function and thereby accelerate atherogenesis. Alternatively, senescence or apoptosis of tissue progenitor cells could, as the primary event, lead to deficient cell replacement/tissue repair and result in decreased function.

Fig. 1. Intracrices can function in their cells of synthesis without secretion (type I), in their cells of synthesis after secretion and reuptake (type II), and in target cells after secretion and internalization (type III). They can also likely function after release in microvesicles and internalization by target cells following fusion of the microvesicles with the extracellular membranes of those cells (type IV). Finally, it may in time be shown that regulatory proteins can transit between cells via nanotubes and function in the recipient cells; if so, this would constitute a distinct form of intracrine action (type V).
Apoptosis is also recognized to play an important role in atherosclerosis. Apoptosis of endothelial cells with cell drop-out occurs in atherosclerosis as does apoptosis of macrophages in atherosclerotic plaque. Macrophage apoptosis is associated with plaque-thinning rupture and can be accelerated by factors such as oxidized LDL. Moreover, EPCs are felt to play a protective or regenerative role in atherosclerosis by restoring lost endothelial cells and restoring endothelial function. The number of circulating EPCs is inversely related to atherosclerosis progression in experimental models; cardiac risk factors such as C-reactive protein, oxidized LDL, and homocysteine increase EPC apoptosis, whereas HDL appears to decrease it (5, 26, 27, 73, 82). Thus there is good evidence to suggest an important pathological role for apoptosis in atherosclerosis and plaque rupture. This then suggests that the mitigation of apoptosis could be important in mitigating atherogenesis and secondarily raises the possibility that intracrines could be used in accomplishing this end.

The intracrine STC is a case in point. As noted above, STC is a secreted hormone that acts at the renal distal tubule to enhance phosphate reabsorption. It is synthesized in bone, prostate, thyroid, ovary, and renal tubules among other sites. STC receptors are found on cell surfaces as well as on inner and outer mitochondrial membranes; STC itself is also associated with these mitochondrial receptors. In the ovary, STC is synthesized as a 50-kDa protein as well as several larger forms, collectively termed big STC. During pregnancy, increasing amounts of big STC are secreted, and as lactation occurs, STC is found in association with the nuclei of the alveolar cells of the breast. As noted above, STC is in some cases internalized by target cells. In addition, STC can be shown to stimulate respiration/electron chain transport in isolated mitochondria from the muscle and liver and in submitochondrial particles from the bovine heart. Collectively, these data show that STC is an intracrine with important effects of phosphate transport that also stimulates mitochondrial respiration in a variety of tissue through a direct intracrine action. STC produced by MSC is antiapoptotic, and it is possible that its actions on mitochondrial respiration and free radicals play a role in this activity. This antiapoptotic action, while thus far demonstrated only in irradiated (i.e., damaged) fibroblasts and hypoxic lung cancer epithelial cells, likely would be beneficial in reducing endothelial cell, cardiac myocyte, and EPC apoptosis. Indeed, human MSCs can inhibit hypoxia-induced apoptosis in cultured aortic endothelial cells, although in this case the intracrines IL-6 and VEGF, possibly in conjunction with other factors, produce the beneficial effect (16, 20, 25, 30, 34, 48, 69).

Similarly, the inability of tissue stem cells as currently used in regenerative therapies to dramatically restore function in damaged myocardium is at least partially caused by the ongoing apoptosis of cardiac myocytes (29, 83). It has been reported that engineered MSCs can express frizzled related protein 2, which much improves their efficacy in mitigating the effects of myocardial infarction and markedly enhances tissue repair by binding Wnt 3a, which, in the context of myocardial infarction, is apoptosis stimulating and is upregulated in ischemic cardiac myocytes (29, 83). It is therefore important to determine whether Wnt is transferred between ischemic cardiac myocytes via microvesicles as well as by more traditional secretory mechanisms. If it is, then attention will have to be paid to inhibiting the trafficking of these microvesicles or blocking the effects of Wnt at the target cell if the regenerative potential of MSCs is to be fully realized. In addition, the possible intracrine action of Wnt proteins, i.e., any action occurring after possible internalization, would have to be taken into account. A similar argument can be made regardless of the microvascular transfer of any other regulatory proteins such as Oct-4, which gain access to the interiors of target cells. Conversely, it will be important to determine whether stem cell-produced apoptotic factors such as STC, which do gain access into target cells, can traffic via microvesicles.

It can also be noted in this context that one member of the Wnt family, Wnt 13, is an intracrine in its own right, and this raises the possibility that other Wnts are as well. Like some other intracrines, Wnt 13 is synthesized in multiple isoforms through the use of alternative promoters, RNA splicing, and alternative translation start sites. One isoform, Wnt 13B, is neither N-glycosylated nor secreted. Rather, it traffics to the mitochondria or nucleus; in the latter case, it stimulates apoptosis in aortic endothelial cells. Although secreted forms of Wnt can be internalized by target cells, it is not known whether there is any trafficking to the nucleus or mitochondria after internalization; an analogy with other intracrines suggests that they may. In any case, Wnt 13 and any microvesicular Wnt that possibly may be transferred into the interiors of cells qualify as intracrines (28, 72, 74). It is also interesting to note that the deficiency of the membrane protein klotho produces a phenotype characterized by premature aging. This is thought to occur for two reasons. First, the absence of klotho prevents the binding of the secreted hormone FGF-23 to target cells with attendant effects on phosphate and vitamin D metabolism, as well as with a variety of age-related pathologies and a shortened life span. Second, the extracellular domain of klotho can be cleaved, leading to its secretion and subsequent binding to Wnt proteins; unchecked Wnt signaling in the absence of klotho leads to accelerated senescence as seen in the klotho-/- mouse. Thus members of the Wnt family, at least one of which is intracrine, interact with senescence and tissue regeneration on multiple levels (40, 46).

Conclusion

The available data indicate that intracrines participate in cellular senescence, apoptosis, and stem cell biology. In some cases (STC), intracrine functionality per se appears to play a role, whereas in other cases, such activity can be suspected but as yet is unproven. Moreover, intracellular regulatory peptides (among other factors) can transit between cells in microvesicles in senescence, stem cell development, and cancer progression. If transferred peptides are not directed to lysosomes or similar structures in target cells, this kind of activity represents intracrine functionality, and although it occurs less frequently than classical intracrine action, it, nonetheless, appears to play a role in cell and tissue biology in health and disease. This phenomenon also complicates attempts to interfere with intracrine function by inhibiting intracrine transit between cells in the extracellular space as, for example, with antibodies (66). Microvesicle-associated intracrines would traffic in a protected intracellular-like space. Rather, microvesicle intracrine action will likely be better inhibited by intracrine blockers that work in the cell interiors of target cells. Although
intracrine action permits the transfer of intracellular regulatory peptide molecules between cells. The exchange of microvesicles and their contents between cells, although occurring less frequently, may accomplish the same result, and as a consequence, the microvesicular trafficking of intracellular regulatory peptides or extracellular-signaling proteins directed to the interiors of target cells can be considered intracrine in nature. Similarly, if peptide intracellular regulators are found to traffic between cells via filaments on interstitial Cajal-like cells, then this, albeit uncommon form of trafficking, should also be considered intracrine, just as the transcription factor trafficking in plants via plasmodesmata is intracrine. The possibility that microvesicular and nanotube trafficking of regulatory factors occurs more frequently in the case of stem cells, which arguably are more closely related to the earliest metazoan cells, than in differentiated cells should be considered. In this context, one can note that gene transfer, not between disparate cells of the same organism but between simple cells of very different genetic backgrounds (species), appears to have occurred earlier in the evolutionary history of life as a consequence of the well-recognized phenomenon of lateral genetic transfer. As a result the genomic evolutionary tree is no longer considered to have a single common trunk (12). Presumably, the transfer of other regulatory factors including peptides occurred during this process as well. Arguably, all of these functionalities, including canonical intracrine action, could be representative of an overarching process characterized by the lateral transfer of genetic- and protein-based information, i.e., a manifestation of what we suggest be termed “laterality.” By “laterality” we imply the transfer between cell interiors of intracellular regulatory factors, be they peptides, miRNA, mRNA, DNA, or other moieties. Thus the intracrine factors that travel between the cell interiors in microvesicles, like those which are secreted and subsequently internalized by target cells, would be considered to operate in a lateral fashion. Intracines that operate in their cells of synthesis but act at target cell membranes and are not internalized by those cells will be deemed to function in an intracrine, but not a lateral, mode; in effect, they represent the crossover point between lateral function and more traditional hormone/paracrine/autocrine peptide action. Although laterality encompasses the transfer of protein, mRNA, miRNA, DNA, and other factors, our interest is in peptide intracrine function and its physiological and therapeutic implications. Nonetheless, our appreciation of intracrine action is arguably informed by an appreciation of the more general principle of laterality.

GRANTS

This work was funded by National Heart, Lung, and Blood Institute Grant R01-HL-072795 and by the Ochsner Clinic Foundation.

REFERENCES


