Scanning Microscopy

Volume 9 | Number 3 Article 11

6-5-1995

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Scanning Microscopy, Vol. 9, No. 3, 1995 (Pages 757-762) 0891-7035/95$5.00+.25
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

CROSS-LINKING OF CELL SURFACE RECEPTORS AS A TRIGGER OF CELL APOPTOSIS AND PROLIFERATION

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(Received for publication January 25, 1995 and in revised form June 5, 1995)

Abstract

A hypothesis of the mechanism by which the protein cross-linking agents trigger apoptosis of lymphoid cells and proliferation of other cell types is proposed. It is assumed that both effects are triggered by aggregation of receptors on cell surface, which results from their cross-linking. This idea is substantiated by the example of one of these agents, ionizing radiation. As in the case of physiological agents, such as, antigens and growth factors, the aggregation of receptors induced by radiation activates receptor protein tyrosine kinases from which the signal is transduced to genes through protein kinase C. The hypothesis is consistent with the relationship between these effects and the PTK-PKC-dependent signal transduction pathway and its activation after irradiation.

Key Words: Apoptosis, proliferation, irradiation, receptors.

Introduction

During the past few years, significant progress has been made in understanding the mechanisms by which physiological agents induce cell proliferation or apoptosis. One of the mechanisms triggering both effects in cells is the aggregation of the receptors whose cytoplasmic domains possess a tyrosine-kinase activity. The cross-linking of extracellular regions of receptors with polyvalent antibodies, and adjacent ligands on the antigen-presenting cell or growth factors activate PTKs of receptors. PTKs, in turn, activate through a cascade of signalling events, the transcription of genes, resulting in proliferation or apoptosis of cells, depending on specificity of cells and agents, as well as on strength of influence. These data are still little used in understanding the mechanisms of induction of these effects by various exogenous agents. The spectrum of these agents is broad and many of them are capable of initiating the cross-linkage of proteins and, consequently, aggregation of receptors. In the present work, it is proposed that this process itself is responsible for initiation of proliferation and apoptosis of cells by agents inducing cross-linking of proteins. This general idea is substantiated by the example of one of these agents, ionizing radiation.

Induction of Apoptosis and Cell Proliferation by Physiological Agents

Apoptosis occurring at a definite stage of differentiation of lymphoid cells is a prerequisite for the normal functioning of the immune system [10, 16]. This process, which is intended for eliminating the autoreactive clones of lymphoid cells [16], has been most intensively studied in maturing thymocytes. It was found that apoptosis occurs in those immature cortical thymocytes ($CD4^+$ $CD8^+$) whose antigenic receptors (TCR) react with autoantigens [16, 33]. For the induction of death of thymocytes maturing in the thymus, a double interaction of the thymocyte with adjacent ligands on the surface of the antigen-presenting cell is necessary, namely, between TCR and the antigen, and between CD4 or

Abbreviations

AP-1 and NF-kB:	transcription factors
GAP:	GTPase-activating protein
LO:	lipoxygenase
PKC:	protein kinase C
PLA_2:	phospholipase A_2
PLC-γ:	phospholipase C-γ
PTK:	protein tyrosine kinase
TCR:	T-cell receptor
RER:	receptor-mediated effect of radiation

CD8 co-receptors and the determinants of the major histocompatibility complex [33, 51]. The fusion of TCR to the co-receptor activates the receptor protein tyrosine kinases (PTK) through their phosphorylation [51]. There is evidence that tyrosine phosphatase CD45 is important in activation of receptor PTKs [19] and hence, a further component may be involved in the activation complex. Apoptosis in immature lymphoid cells can be induced not only by the determinants of the antigen-presenting cells but also by the antibodies to receptors [33, 48, 55]. It was shown that, in this case, apoptosis is also initiated by receptor aggregation [29, 45] which activates receptor PTKs [49, 55].

The receptor PTK activation, which results in death of immature thymocytes, triggers the proliferation of mature T-cells [1]. Proliferation of other cell types (e.g., epidermal, fibroblast) is stimulated by various growth factors (e.g., EGF, FGF) [9]. The process is initiated by the aggregation of growth factor receptors followed by the activation of receptor PTKs through their reciprocal phosphorylation at tyrosine [9, 34, 43]. Thus, both apoptosis of immature thymocytes and proliferation of other cell types that occur in response to physiological agents, are triggered by receptor aggregation which causes the activation of PTKs.

The pathway of signal transduction from receptor PTKs to the genes whose products are directly responsible for the stimulation of cell proliferation has been examined in detail in experiments involving growth factors. Several signalling molecules (e.g., PLC-γ, GAP, etc.) [9, 34] bind to the regions of the receptor PTKs phosphorylated at tyrosine to be in turn phosphorylated at tyrosine [9, 55]. The signal is then transduced through these molecules to PKC. There are three possible pathways of signal transduction from receptor PTKs to PKC: (1) Through PTK-dependent activation of PLC-γ [24, 38]. This enzyme hydrolyses phosphatidylinositol-bisphosphate to yield PKC-activating messengers diacylglycerol and inositoltriphosphate [4, 26, 31]. (2) Through PTK-dependent increasing in the concentration of the active form of Ras, Ras-GTP, which stimulates PKC [12, 17]. The increase in the Ras-GTP concentration is likely to be due to the PTK-dependent inhibition of the activity of the enzyme that hydrolyses Ras-GTP (GAP). As noted above, GAP is one of the molecules that bind to the activated receptor PTKs. (3) Through activation of PLA_2 which is achieved by tyrosine phosphorylation either of the enzyme itself [35] or of lipomodulin which inhibits PLA_2 in the non-phosphorylated state [6]. The products of PLA_2, such as, lysophospholipids, arachidonic acid and the metabolic products of the lipoxygenase pathway of its metabolism (leukotrienes), activate PKC, both directly [13] and indirectly, by inhibition of GAP [12, 46]. The necessity of PKC and PLA_2 interaction for apoptosis is also shown in our current work [41]. PKC transduces the signal to different genes by activating the transcription factors (e.g., AP-1, NF-kB) [2, 17]. Figure 1 schematically presents the described sequence of signalling events, it clearly shows that PTK and PKC are the obligatory participants of signal transduction. The signal transduction from PTK to PKC may proceed through different ways, and the contributions of these pathways may vary among different cell types [14].

In immature thymocytes, the sequence of signalling events in induction of apoptosis by physiological agents has been less studied, however, the available information suggests that activation of PTK and PKC is also a mandatory step in this process [1, 29, 55].

Thus, cell death by apoptosis and cell proliferation are initiated by aggregation of receptors, and PTK and PKC are the obligatory components of signal transduction to genes. The latter distinguishes this signal transduction pathway from other signalling systems. For instance, PTK is not involved in signal transduction from the receptors bound to G-proteins [11], and signal transduction occurring through K^+ channels or via DNA injuries is independent of both PTK and PKC [2, 22].

A Possible Mechanism of Activation of PTK-PKC-dependent Signalling Pathways by Ionizing Radiation

The ionizing radiation exerts diversified effects on mammalian cells. Induction of some of these effects requires activation of genes and synthesis of macromolecules. Among these are proliferation stimulated by low radiation doses [15, 25], and radiation-induced apoptosis in lymphocytes [39]. Since these effects of radiation are mediated via active cell metabolism, irradiation is here not a direct cause of the effect but serves as a signal, triggering a series of events, that just result in the observed phenomena. Recent studies have demonstrated

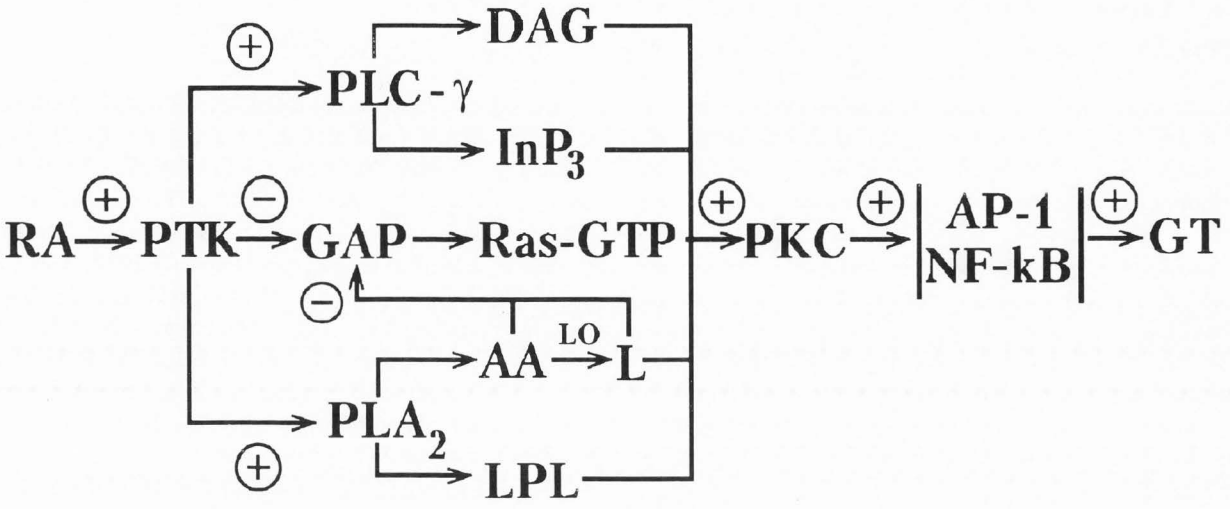

Figure 1. Schematic diagram showing the signal transduction pathway from receptors to genes upon activation of cell proliferation by growth factors. The abbreviations used here are: RA: receptor aggregation; GT: gene transcription; DAG: diacylglycerol; InP_3: inositoltriphosphate; LPL: lisophospholipid; AA: arachidonic acid: L: leukotriene; ⊕: activation; and ⊖: inhibition.

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that irradiation with doses (0.1-1 Gy) causing apoptosis of lymphoid cells and stimulating division of other cell types, activates the transcription of the same genes as physiological stimulus do. In particular, irradiation activates the transcription of c-jun [52], c-myc [8] and the gene of IL-1 [15, 53]. The transcription of these genes is PKC-dependent [18], and after irradiation, the PKC activity in cells increases several fold [18, 25, 54]. Irradiation also activates PTK as evidenced by tyrosine phosphorylation of some proteins in irradiated cells [48], with the radiation-induced activation of PKC being dependent on PTK [47]. These findings indicate that ionizing radiation switches, in some way, the system that transduces signals from the growth factor and antigen receptors, resulting normally in stimulation of cell proliferation and apoptosis of immature cortical thymocytes. Currently, there is direct evidence that the PTK-PKC-dependent signalling pathway plays a crucial role in radiation-induced apoptosis of lymphoid cells. In particular, it was found that the inhibitors of PTK and PKC effectively prevent DNA fragmentation in irradiated lymphoid cells [32, 47, 48]. The absence of increase in Ca^{2+} concentration in thymocytes within the first hour after exposure [40], and the almost complete suppression of apoptosis by the inhibitors of PLA_2 and LO [20], suggest that the radiation-induced signal is not transduced to PKC via PLC-γ, a pathway leading to the increase in intracellular Ca^{2+} concentration, but rather via PLA_2, and possibly, Ras-proteins (Fig. 1).

The PTK-dependence of radiation-induced apoptosis suggests that it is triggered by activation of PTKs. Since the aggregation of receptors is a natural way of receptor PTK activation, it would be logical to assume that during irradiation, PTKs would also be activated by this mechanism. The aggregation of receptors during irradiation should be accomplished by any covalent cross-links arising from recombination of protein radicals. The cross-links between the growth factor receptors may initiate proliferation of mature T-cells and other cell types, whereas the cross-links between T-cell receptors and CD4 or CD8 may result in apoptosis of cortical thymocytes. The principal possibility of triggering cell proliferation by protein cross-links was shown in the analysis of lymphocyte blast transformation in response to periodate [5]. In these experiments, the blast transformation was due to membrane protein cross-links of the Schiff-base type (C = N) [5]. It seems likely that the proliferation was initiated in those cells in which cross-linking between the growth factor receptors took place.

The growth factors and antigens are efficient at very low concentrations. In particular, the concentration for the antigen to induce, *in vitro*, the positive selection of thymocytes is 10^{-12} M [37]. It is believed that the cell may be activated by one antigen [7], i.e., by the formation of one receptor cluster. Thus, the number of receptors needed for cell activation is as little as a few hundredths of a per cent of the total number of receptors of a given type reaching tens of thousands per cell [36].

The presented estimates indicate that the radiation-induced formation of receptor aggregates sufficient to change the functional state of cells, is difficult to measure directly. To do this, it is necessary to increase the dose to a level allowing reliable determination of the desired product. The yield of cross-links was estimated for major erythrocyte membrane proteins, spectrin and capnophorine, in erythrocyte ghosts irradiated with doses of hundreds of Gy [42]. It was shown that the amount of cross-links increases linearly with dose, reaching, under aerobic conditions, 20% of the total amount of the membrane protein at a dose of 200 Gy [42]. An extrapolation of this linear dependence on low doses yields that after a 0.1 Gy dose the output of cross-links will account for a few hundredths of a per cent of the total amount of the protein, which is sufficient for triggering the signalling system.

The regularities of cross-linking of membrane proteins in irradiated cells have been revealed in studies on erythrocyte ghosts as well [23, 42]. It was shown that a major outcome of irradiation was protein aggregates. This effect was due mainly to OH^+ radicals, that is, the cross-links were an indirect effect of irradiation. Oxygen reduced the radiation-induced yield of cross-links in irradiated erythrocyte ghosts. The effect of oxygen on cross-linking of proteins in biological membranes during irradiation may be masked by lipid peroxidation products whose amounts are known to increase in the presence of oxygen [50], and which are capable of initiating protein cross-links [21, 50]. If lipid peroxidation is involved in aggregation of receptors, then, it would be difficult to predict how oxygen would affect the receptor-mediated effects of radiation (RER). In support of the involvement of lipid peroxidation in induction of RER is the finding that the inverse dose rate-effect relationship, which is typical of lipid peroxidation [44], is also observed for RER, such as, apoptosis of thymocytes [27] and activation of PKC-dependent genes [52].

Soszynsky and Schluessler [42] reported that S-S links made a significant contribution to aggregation of the integral membrane protein capnophorin. Since many receptors contain domains rich in SH groups [9], they may also aggregate because of the formation of disulfide bonds. These bonds may arise not only from the reaction with OH^+ but also with hydrogen peroxide as well [3]. Hydrogen peroxide is a stable product of water radiolysis, and if S-S bonds are involved in the aggregation of receptors, then RER may be initiated by the hydrogen peroxide arising in irradiated medium. Indeed, some observations indicated that irradiated medium [30] and hydrogen peroxide [28] do stimulate cell division.

Thus, the hypothesis of initiation of RER by cross-linking of receptors does not contradict the available data, thereby, allowing its experimental verification in several cell lines.

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Discussion with Reviewers

E. Falcieri: Does the author think that the phenomenon of radiation-induced receptor aggregation may be an acute or long-term effect?

Author: Receptor aggregation may be initiated during the time of irradiation (acute) and after irradiation by radiation-induced hydrogen peroxide or lipid peroxide (long-term).

E. Falcieri: Could it be plausible a chemical modulation of this phenomenon and a certain adaptation to repeated exposure?

Author: This phenomenon may be modified with S-S-reducing agents, lipid peroxidation inhibitors, oxidants, inhibitors and activators of PTKs, PLA_2, LO and PKS. There are some data in this field in the literature [5, 28, 32, 41, 50, 54, 55].

A certain adaptation to repeated exposure is possible because the first dose switches signalling system and the cells are changed before second dose.

W. Leyko: Which techniques and methods could be used to state the eventual cross-linking of cell surface receptors following 0.1-1 Gy doses of irradiation?

Author: The methods may be indirect (the correlation with chemical modification of receptor cross-links with the magnitude of RER) and indirect (the decreasing of SH-groups in membrane proteins, and the decreasing of ligand adsorption on cell surface).