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Review

The complex understanding of Annexin A1 phosphorylation ☆

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ABSTRACT

Annexin A1 (ANXA1) is the first characterized member of the annexins superfamily. It binds the cellular membrane phospholipids in Ca^{2+} regulated manner. Annexin A1 has been found in several tissues and many physiological roles as hormones secretion, vesiculation, inflammatory response, apoptosis and differentiation have been shown. Its subcellular localization and binding with many partner proteins are altered accordingly with its physiological role. The Annexin A1 membrane localization is crucial for binding to receptors, suggesting a paracrine and juxtacrine extracellular action. Annexin A1 is subjected to several post-translational modifications. In particular the protein is phosphorylated on several residues both on the N-terminal functional domain and on the C-terminus core. Different kinases have been identified as responsible for the phosphorylation status of selective residues. The specific change in the phosphorylation status on the different sites alters ANXA1 localization, binding properties and functions. This review shows the physiological relevance of the ANXA1 phosphorylation leading to the conclusion that numerous and different roles of Annexin A1 could be associated with different phosphorylations to alter not only intracellular localization and bindings to its partners but also the extracellular receptor interactions.

Contents

1. Introduction

About 30 years ago, a 37 KDa protein was identified as a steroid-induced inhibitor of phospholipase activity with potential anti-inflammatory action. The protein was named lipocortin-1, lipomodulin, macrocortin or renocortin. Currently it is mostly known as Annexin A1 (ANXA1).

It is phosphorylated in vivo by protein tyrosine and serine–threonine kinases. During the years, many physiological functions

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related to ANXA1 activity, localization and modifications have been described. It was found that ANXA1 inhibits the phospholipase and it has been proposed that this activity could be regulated by its phosphorylation. In 1986 Pepinsky & Sinclair described the phosphorylation of Annexin A1 near its amino terminus by the protein tyrosine kinase activity of the epidermal growth factor receptor (EGF-R) [1]. This study has been updated and amplified by using recombinant Annexin A1 as a substrate for several putative protein kinases. The authors identified several phosphorylated residues by a combination of peptide mapping and sequence analysis and showed that recombinant pp60c-src phosphorylates Annexin A1 near its amino terminus, at tyrosine 21 (Tyr21). Also polyoma virus middle T/pp60c-src complex, recombinant pp50v-abl, and the EGF receptor/kinase phosphorylated the same tyrosine residue. It was also shown that serine 27 residue of ANXA1 is the primary site phosphorylated by protein kinase C (PKC). In the same study, the threonine 41 residue has been identified as a PKC substrate as well. The adenosine cyclic 3′,5′-phosphate dependent protein kinase A (PKA) phosphorylates ANXA1 in its carboxyl-terminal core at the threonine 216 residue (Thr216) [2]. In 1988 Schlaepfer & Haigler showed that PKC phosphorylated N-terminal peptides cleaved from ^{32}P-labeled AnnexinA1 contain phosphorylated threonine-24, serine-27, and serine-28 [3]. These studies raised the attention focus on the ANXA1 phosphorylation status and its possible role in regulation of the Annexin A1 functions (Fig. 1).

2. Initial studies of the phosphorylated Annexin A1 physiological roles

The annexins are a family of calcium-dependent, phospholipid-binding proteins [4]. Annexin A1, as the other annexins, is activated in its Ca^{2+}-bound conformation to bind the membrane phospholipids due to the exposure of its functional N-terminal domain. In contrast, the Ca^{2+}-free ANXA1 N-terminus is sterically hindered and not accessible [5]. Most of the ANXA1 functions are related to its ability to interact with cellular membranes. This interaction is reversible and regulated especially by post-translational modifications like phosphorylation. Tyrosine phosphorylation decreases the ANXA1 Ca2+ requirement for binding phosphatidylserine vesicles [6]. In 1992 Patte et al. published that treatment of synchronized HUVE endothelial cells with basic Fibroblast Growth Factor (bFGF) leads to tyrosine phosphorylation of Annexin A1. According to the published results, the effect of bFGF on ANXA1 phosphorylation occurs only during S phase of the cell cycle, suggesting a role of the FGF-receptor/kinase complex [7].

Wang et al. showed that phosphorylation of the N-terminal domain of ANXA1 by PKC induces chromaffin granules aggregation, responsible for the secretion of hormones. Their study started with the evidence that phosphorylation of serines of ANXA1 is induced by bovine chromaffin secretion [8]. According to their results, the phosphorylation by PKC of an unspecified residue leads to a strong inhibition of ANXA1 aggregative ability. The mechanism of action is probably related to the fourfold increase of Ca^{2+} requirement. The inhibitory effect was reversed by the phosphatase 2A mediated dephosphorylation of the Annexin A1. On the other hand, the ANXA1 phosphorylation by PKC was not able to interfere with the direct binding of ANXA1 to granules or phosphatidylserine and phosphatidylcholine vesicles, leaving unexplained the inhibitory effect on ANXA1. For this reason, the authors suggested, in an articulated discussion, an "unusual mechanism" of inhibition involving a sort of not yet established competition mechanism between the phosphorylated and unphosphorylated ANXA1 forms. Moreover the authors proposed a role of ANXA1 in regulation of hormones exocytosis via the ability to alter granules aggregation. [9] However, the process must be further characterized to drive a final conclusion.

At the same time it was shown that low concentrations (5 μM) of glycyrrhizin and a glycyrrhetinic acid derivative induced a protein kinase A (PKA) mediated ANXA1 phosphorylation. Curiously, higher concentrations of these compounds reverted completely the effect. As the natural compound examined showed anti-inflammatory activity, the conclusion and the speculation were that ANXA1 phosphorylation may have a role in the anti-inflammatory effects of the studied drugs [10].

Sawyer and Cohen showed that the ANXA1 phosphorylation is linked with EGF-R internalization suggesting that the endosomal EGF-R kinase may be involved in the phosphorylation of Annexin A1 [11]. Based on their results, it was shown by Futter et al. that ANXA1 is a substrate of EGF-R kinase in multivesicular bodies (MVBs, important for stimulated EGF-R internalization and sorting). Interestingly, phosphorylating a calcium-independent Annexin A1 converted it to a calcium-dependent form. The ANXA1 phosphorylation has been suggested being responsible for the MVBs inward vesiculation induced by EGF-R activation. The phosphorylated ANXA1 residue in this study is not shown. Other experiments were performed using both in vitro and cell culture approaches [12]. Summarizing the results and recent literature covering this topic, it can be concluded that ANXA1 is phosphorylated by EGF-R after internalization via the multivesicular bodies. This phosphorylation is not calcium-dependent but the phosphorylated Annexin A1 binds the phospholipids in a calcium-dependent manner.

Protein Kinase C (PKC) catalyzed phosphorylation of Annexin A1 and 2 in cultured rat mesangial cells in vitro required presence of Ca^{2+} and phospholipids. However, mere phospholipids were not sufficient to support the phosphorylation. In the experiments performed in vivo on ^{32}P-labeled mesangial cells, the ANXA 1 phosphorylation was increased by the addition of PKC activators as angiotensin II and phorbol myristate acetate (PMA). Moreover, a phosphoamino acid analysis, either by using two-dimensional chromatography or specific antiphosphotyrosine antibody, revealed that phosphorylation occurs only on serine residues [13]. These results were updated and partially confirmed by a study performed on rat renal glomeruli and rat

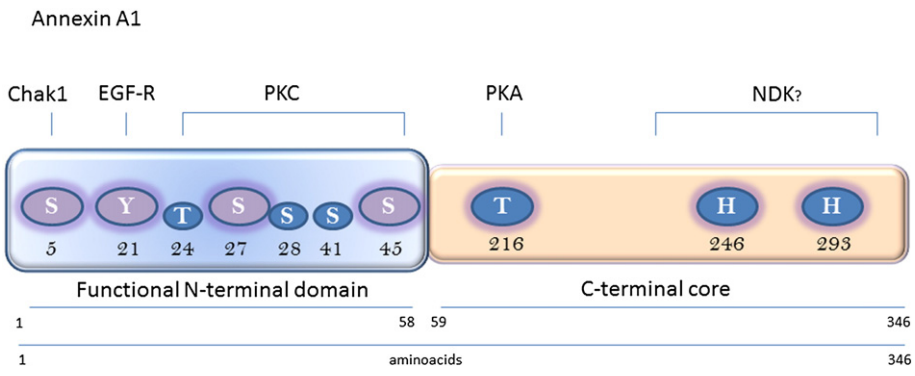

Fig. 1. Schematic representation of the target residues and principal kinases involved in ANXA1 phosphorylation. S, Y, H and T are respectively Serine, Tyrosine, Histidine and Threonine. Chak1 — TRPM7 Channel Kinase 1; EGFR — Epidermal Growth Factor Receptor; PKC — Protein Kinase C; PKA — Protein Kinase A.