Original Article

ROC1 Protein, Cell Cycle and Cancer

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Abstract

Enhanced proliferation and increased survival are important characteristics of neoplastic cells. Ubiquitination and subsequent degradation by regulatory proteasome´s enzymes controls several biological processes, including entry into the cell cycle, transcription and signal transduction. The proteolysis of many regulatory proteins in G1 phase is mediated by the ubiquitin-ligase complex SFC (SKP1-CUL1(CDC53)-F-box) which is composed by four major subunits (Skp1, Cul1, Rbx1/ROC1) and of many F-box proteins. The ROC1 proteins are RING finger proteins from ubiquitin-ligase E3 family. They function as a central component in the ubiquitination pathway, not only participating in the transference of ubiquitin-conjugating enzyme E2, but also being responsible for the reaction specificity. Mutations in one amino acid in ROC1 protein domain are able to completely inactivate ubiquitin-ligase activity, which suggests that this domain is essential for this activity, and consequently essential for control of cell cycle proteins. Therefore, mutations can cause loss in control of cell cycle regulation, which leads to enhanced proliferation and increased survival of neoplastic cell.

Keywords: Neoplams. Cell Cycle. ROC1 Protein. Ubiquitin.

Introduction

Cell proliferation regulation is the key event in normal development, in the responses to cell aggression and in tumorigenesis. Cells orderly progression of the cells through cell cycle regulation depends on the balance between the concentrations of activated cyclin and Cyclin-dependent kinases.[1]

Both genic amplification and the increase of proteic stability of cell cycle regulatory proteins may cause the latter's superexpression in tissues[2] and both mechanisms have been associated with oncogenesis in different types of human cancers.[3]

Degradation of cell cycle G1 phase regulatory proteins is as important for the end of mitosis as is their synthesis for the beginning of mitosis. Many of these proteins are destroyed quickly by proteolysis in the transition metaphase-anaphase. This process requires a sequence-sign in the polypeptidic chain of the protein to be destroyed, making it to degrade by supplying ubiquitin a ligation site.[2] Thus, these proteins proteic stability is normally regulated by ubiquitin-dependent proteolysis.[3]

Ubiquitin-Mediated Proteolysis

Most proteins degraded in cytosol are transferred to great protein complexes called proteasomes, which are present in many copies in the entire cell. Each proteasome consists of a central cylinder, formed by multiple different proteases in its inner surface and great

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protein-blocking complexes in the outer. Proteasomes act on proteins targeted to destruction by a covalent link of a small protein called ubiquitin.[2]

Ubiquitin-dependent proteolysis and subsequent degradation by regulatory enzymes' proteasome control proteic stability and has an important role in the regulation of several biological processes, including entry into the cell cycle, transcription and signal transduction.[4-8]

Different ubiquitin-dependent proteolytic pathways use enzymes conjugated to different ubiquitins, which are structurally similar, associated to subunits of recognition of proteins targeted by a particular sign of degradation. The enzyme that, when conjugated, adds ubiquitin to a residue of lysine of a target-protein, and subsequently, adds a series of additional ubiquitins, forming a polyubiquitin chain that is recognized by a specific receptor protein in proteasomes.[2,9] Polyubiquitin chains are linked covalently to the target-protein through a cascade of three enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin-protein ligases (E3). Acting in the last stage of cascades, ubiquitin-protein ligases (E3) work as a central component of ubiquitination pathways, catalyzing the final transfer of ubiquitin from E2 to the substrate, and governing the specificity and the time of the modified reaction (Figure 1).[4-5,7-10.]

Ring Proteins

The interaction of E2 and E3 proteins is done by proteic fragments called RING proteins, evolutionarily conserved structures found in more than 200 proteins, in which loops of two amino acids, are pulled together at their base by eight cysteines or histidine residues that bind to two zinc ions. Many RING proteins participate in ubiquitination and are essential for ubiquitin-ligase activity.[12]

There are many RING proteins in all eukaryotes and more than 350 in human genome. Both the length and sequence of cysteines and histones vary significantly in the different RING proteins. Depending on the arrangement of cysteine and histidine residues, RING proteins may be classified in three subclasses: C3H2C3 (or RING-2), C3HC4 (or RING-HC) and less frequently C2H2C4.[13]

The SCF Complex

SCF protein (SKP1-CUL1(CDC53)-F-box) and Anaphase Promoter Complex (APC) are the two major ubiquitin-ligase complexes, regulating ubiquitin-mediated proteolysis during G1/S phase and anaphase, and they contain the small RING ROC1 and APC11 proteins, respectively.[3,14-16] SFC ubiquitin-ligase complex is composed by four major subunits, Skp1, Cul1, Rbx1/ROC1 and one of the many F-box proteins (Figure 2).[14]

SCF ubiquitin-ligase proteins include a member of the family of F-box proteins (so called because cyclin F was one of the first proteins in which this fragment was identified), which serve to recognize and to recruit target-proteins.[17] F-box proteins bind to the module CUL1/ROC1 by the adapter protein Skp1 (S-phase kinase associated protein-1).[10,18-19] The cullins (CUL) are a family of evolutionarily conserved proteins that assemble a large family of cullin-dependent E3 ligases (CDL). All cullins have a carboxyl-terminal domain of about 100 amino acids, which binds to the small RING ROC1 and APC11 proteins.[4,20]

Cand1 protein controls SFC complex formation, inhibiting it, preventing Skp1 and of F-box proteins

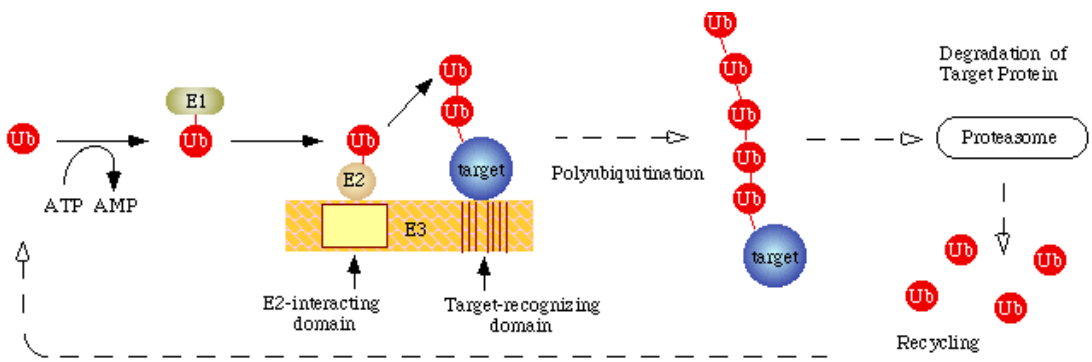

Figure 1 – Ubiquitin-mediated proteolysis (Source: www.genome.jp/kegg[11])
*target: target-protein to be degraded.

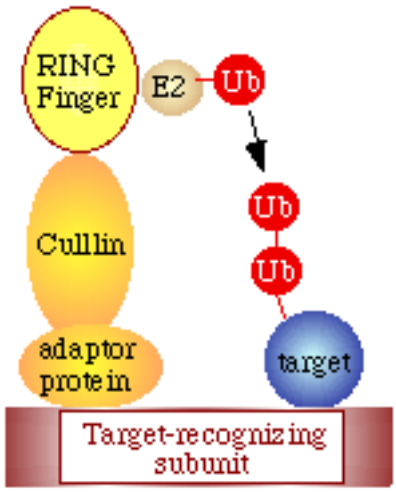

Figure 2 – Scheme of SCF complex targeting protein for proteolysis (Source: www.genome.jp/kegg[11])
*target-recognizing subunit: F-box protein; adaptor protein: Skp1 protein; RING finger: ROC1 protein; target: target-protein to be degraded.

access of the to CUL1-ROC1 fraction, thus avoiding ubiquitination of F-box proteins. Cand1 binds to CUL1-ROC1 fraction and it inhibits the access to E3 complex.[5,21-27]

ROC1 Protein

ROC1 RING protein (RING of Cullins), also called Rbx1 and Hrt1, is quite stable, being an essential subunit of ubiquitin-ligase SCF protein.[3,13] It belongs to the C3H2C3 (or RING-2) subclass of RING proteins. It was first isolated in levedures[28] and purified biochemically as a common component of SCF complex of humans and levedures6,[29-30] as well as of von Hippel-Lindau tumor-suppressor complex (CBCVHL or Cul2-Elongin BC-VHL).[10,31]

ROC1 protein is encoded by human gene Rbx1, composed of five exons, located in chromosome 22q 13.[32] Mutations in only one amino acid in ROC1 protein domain are able to completely inactivate ubiquitin-ligase activity.[3,13,28,33] It is a mediator of proteic substrates degradation required for cell cycle progression, signal transduction and tumor-suppressing activities.[31] In contrast to APC11 protein that only interacts with cullin/APC2 protein, ROC1 interacts to cullin (CUL) 1, CUL2, CUL3, CUL4A and CUL4B, and functions as an adapter of ubiquitin-conjugating enzyme E2, amplifying its role in ubiquitination.[6,13,28-29,31,34] ROC1 has two domains, a C-Terminal able to activate UBC5-E2 and a N-Terminal that interacts to CUL1, which by its turn will ligate to an E2.[13]

ROC1 Protein And Cell Cycle Proteins

NF-kappa B (NF-kB) transcription factor activation has an anti-apoptotic role in the response to cell damage. Stimuli that normally activate NF-kB pathway include Interleukin (IL)-1, tumor necrosis factor (TNF) - a,lipopolysaccharides, hypoxia/reoxygenation[35] and the apoptosis-inducing ligand related to tumor necrosis factor (TRAIL).[36] This requires phosphorylation and ubiquitin-dependent degradation of the NF-kappa B inhibitor, I kappa B alpha (IkB).[35-37] Phosphorylated IkB may be recognized by F-box ^{HOS} protein[38-40] and is flagged to degradation by the proteasome, which recruits for this SCFHOS-ROC1 ubiquitin ligase E3.[4,28-29,38,41]

Cytokines of transforming growth factor (TGF)-B family are multifunctional proteins that regulate growth, differentiation, apoptosis and morphogenesis of several cell types. TGF-β antagonizes mitogenic activity of many other growth factors interfering with cell cycle progression1. Smads are the central mediators of TGF-β family.[15] TGF-β family is regulated by ubiquitin-mediated degradation. Both Smad concentration in non-stimulated cells and Smad protein levels after TGF-β pathway activation are controlled by ubiquitination. Ubiquitin-ligase E3 associated to Smad degradation are Smad ubiquitination-related factor 1 (Smurf1), Smurf2, Smurf3 and the SCF/ROC1 complex.[15,43-44] Abnormalities in ubiquitin-ligase E3 that control TGF-β family components may cause the development and progression of several cancer types.[44]

Cyclin D1 decodes the regulating subunit of a holoenzyme, which phosphorylates and inactivates retinoblastoma protein (pRB), promoting progression to cell cycle G1/S phase.[1] Cyclin D1 exaggerated amplification or expression has an important role in tumorigenesis[45] in several human cancers, including breast cancer, lymphomas, melanomas and colorectal cancer.[46] Studies have shown the role of SCF-ROC1 protein in cyclins D1, D2 and D3 in humans, proving the important role this protein has in cyclin-proteolysis regulation.[3,46-47]

The entry of cells in phase S of cell cycle depends on the degradation of cyclin-dependent kinase (CDK) inhibitor, p27 protein, at the end of phase G1 through ubiquitination pathway, which demands first its phosphorylation and subsequent recognition by SKp2 protein and SCF-ROC1 complex, for it to be degraded.[49-53] Decrease of p27 due to the increase of its degradation is correlated to more aggressiveness and poor prognosis in human epithelial tumors and lymphomas.[14,54-55] The weakening of the interaction of histones and DNA, which makes possible the recruitment of repair proteins, facilitates DNA damage reparation. Biochemical studies indicate that CUL4-DDB-ROC1-mediated histone ubiquitination weakens the interaction of histones and DNA.[56]

DNA synthesis depends on prereplicating complex, which has an essential component CDT1 (Cdc10-dependent transcript 1). The increase of CDT1 expression is associated to replication induction and polyploidie.[57] CDT1 is degraded by ubiquitin-dependent proteasomal pathway during cell cycle S phase. In mammals, CDT-1 degradation induced by DNA damage is catalyzed by CUL4-DDB1-ROC1 ubiquitin E3 ligase.[58-61] CDT1 levels fall immediately after gamma or ultraviolet (UV) irradiation and the interaction between CDT1 and CUL4-DDB1-ROC1 is in part regulated by gamma irradiation.[62] CDT1 constitutive expression is associated to the development of a subgroup of lung non-small cell carcinoma in humans, indicating the critical importance of CDT1 levels to the beginning of DNA replication and maintenance of genome integrity.[63]

p53 protein has a role in inhibiting cell proliferation and apoptosis triggering. It obstructs neoplastic transformation using three mechanisms: activating cell cycle temporary interruption, inducing cell cycle permanent interruption or promoting apoptosis. Aggressions to genome cause an increase of p53 levels, which combines with transcription factors for preventing the cell from entering in the S phase and allowing repairing of DNA damage and the reestablishment of genome integrity. If DNA alterations exceed repairing capacity, p53 protein unleashes cell death by apoptosis.[64] One of the first references to ubiquitin-mediated p53 protein proteolysis was done in a study of the way human papillomavirus (HPV) types 16 and 18 protein E6 inactivates p53. Cell protein E6-AP, an ubiquitin-ligase E3, interacts with protein E6 of HPV, forming the E6/E6-AP complex, which ligates and flags p53 protein for ubiquitin-mediated proteolysis Many evidences suggest that this activity has an important role in the HPV-associated carcinogenesis.[65] Protein E1B-55K of adenovirus type 5 neutralizes in the first phase of infection p53 protein activity, which may adversely affect viral replication or promote host cell apoptosis66-67. One of the mechanisms by which protein E1B-55K neutralizes p53 protein is acting with protein E4-orf6 for stimulating p53 degradation by proteasomes.[66-67] It was shown that cellular factors, including CUL7, CUL5, Elongin B, Elongin C and ROC1 protein interact with E1B-55K and E4-orf6 and cause p53 ubiquitination and promote their degrading.[66-68]

The Detection of ROC1 Protein in Tissues

ROC1 protein can be detected by using epitope-specific rabbit polyclonal antibody for ROC1. It is a synthetic peptide derived from the C-Terminal portion of human ROC1 protein that may be used to Western Blot, immunoprecipitation, ELISA and immunohistochemistry in formalin-fixed and paraffin-embedded tissues.[69]

In immunohistochemical assays, ROC1 protein has a nuclear staining and the squamous epithelium of tonsil, and HeLa cells can be used as positive controls for reactions.[69] (Figure 3)

General Comments

ROC1 RING protein has a critical role in ubiquitination and is thus vital for controlling proteins involved in cell cycle. Mutations in the gene that encodes ROC1 may cause loss of control of cell cycle regulation, favoring tumor cells quick proliferation and increasing their survival. Studies on the possible role of this protein in the origin of human cancers are still necessary, principally in cases where there is cell cycle proteins overexpression without alteration of genic expression.

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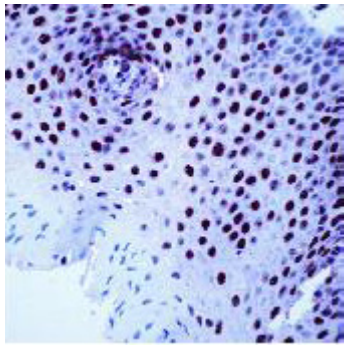

Figure 3 - Nuclear immunostaining with ROC1 antibody in squamous epithelium of tonsil (Source: www.labvision.com)[70]

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