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Review article
Pregledni članak

MARP PROTEIN FAMILY: A POSSIBLE ROLE IN MOLECULAR MECHANISMS OF TUMORIGENESIS

PROTEINI FAMILIJE MARP: MOGUĆA ULOGA U MOLEKULARNIM MEHANIZMIMA TUMOROGENEZE

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Summary: The MARP (muscle ankyrin repeat protein) family comprises three structurally similar proteins: CARP/Ankrd1, Ankrd2/Arpp and DARP/Ankrd23. They share four conserved copies of 33-residue ankyrin repeats and contain a nuclear localization signal, allowing the sorting of MARPs to the nucleus. They are found both in the nucleus and in the cytoplasm of skeletal and cardiac muscle cells, suggesting that MARPs shuttle within the cell enabling them to play a role in signal transduction in striated muscle. Expression of MARPs is altered under different pathological conditions. In skeletal muscle, CARP/Ankrd1 and Ankrd2/Arpp are up-regulated in muscle in patients suffering from Duchene muscular dystrophy, congenital myopathy and spinal muscular atrophy. Mutations in *Ankrd1* gene (coding CARP/Ankrd1) were identified in dilated and hypertrophic cardiomyopathies. Altered expression of MARPs is also observed in rhabdomyosarcoma, renal oncocytoma and ovarian cancer. In order to functionally characterize MARP family members CARP/Ankrd1 and Ankrd2/Arpp, we have found that both proteins interact with the tumor suppressor p53 both *in vivo* and *in vitro* and that p53 up-regulates their expression. Our results implicate the potential role of MARPs in molecular mechanisms relevant to tumor response and progression.

Keywords: MARPs, p53, tumorigenesis

Kratak sadržaj: Familiju MARP (muscle ankyrin repeat proteins) čine tri strukturno slična proteina: CARP/Ankrd1, Ankrd2/Arpp i DARP/Ankrd23. Sva tri proteina poseduju ankirinske ponovke preko kojih ostvaruju protein–protein interakcije kao i signal za lokalizaciju u jedru. Članovi familije MARP imaju strukturnu i regulatornu funkciju i mogu biti lokalizovani i u jedru i u citoplazmi mišićne ćelije. Učestvuju u signalnoj transdukciji kao molekulski glasnici koji prenose informacije mehaničkog stresa sa sarkomere do jedra, gde učestvuju u regulaciji genske ekspresije. Nivo proteina CARP/Ankrd1 i Ankrd2/Arpp je izmenjen u mišićnim bolestima koje karakteriše atrofija mišića, kao što su Dišenova mišićna distrofija, kongenitalna miopatija i spinalna mišićna atrofija. Mutacije u genu za CARP/Ankrd1 su otkrivene u pacijenata sa dilatiranom i hipertrofičnom kardiomiopatijom. Promene u ekspresiji ovih proteina su takođe uočene u tumorima kao što su rabdomiosarkom, onkocitom bubrega i kancer ovarijuma. U cilju funkcionalne karakterizacije proteina familije MARP, pokazali smo da oba proteina interaguju sa supresorom tumora p53, a geni za CARP/Ankrd1 i Ankrd2/Arpp su pozitivno regulisani ovim transkripcionim faktorom. Rezultati su ukazali na moguću ulogu proteina CARP/Ankrd1 i Ankrd2/Arpp u molekularnim mehanizmima tumorogeneze, čime se otvara novo polje istraživanja ove familije proteina.

Ključne reči: MARP, p53, tumorogeneza

Introduction

Cardiac ankyrin repeat protein (CARP/Ankrd1) and ankyrin repeat domain 2 (Ankrd2/Arpp), together with diabetes associated ankyrin repeat protein (DARP), belong to a conserved muscle ankyrin repeat protein (MARP) family (1). They share approximately 50% homology and contain tandem ankyrin repeats, the coiled-coil domain, nuclear localization signal (NLS), PEST protein degradation sequence and numerous potential modification sites,

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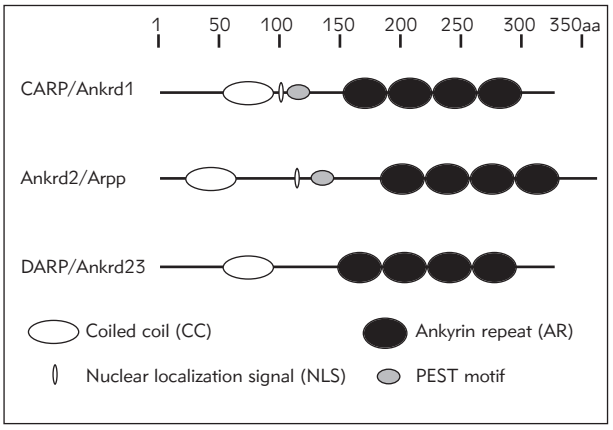

Figure 1 Domain organization of muscle ankyrin repeat protein family members. Schematic representation of human cardiac ankyrin repeat protein (CARP/Ankrd1), ankyrin repeat domain 2/ankyrin repeat protein with a proline-rich region (Ankrd2/Arpp) and diabetes related ankyrin repeat protein (DARP/Ankrd23) sequences indicating the presence of the coiled-coil domain, nuclear localization site (NLS), ankyrin repeats and PEST motif.

mainly for phosphorylation (*Figure 1*). Ankyrin repeats are ubiquitous motifs involved in protein–protein interactions, whereas PEST sequences serve as signals that target proteins for rapid destruction. The MARPs are preferentially localized in cardiac and skeletal muscle and their expression is induced under stress conditions. They are found in differing abundance in the central I-band of the sarcomere, where they bind the N2A region of titin and the amino-terminus of the nebulin anchoring protein myopalladin (1, 2). CARP/Ankrd1 and Ankrd2/Arpp shuttle to the nucleus where they participate in the regulation of gene expression, serving as mediators between the stress and transcriptional response. In this paper we revise the current knowledge on the expression profiles, regulation and function of MARP family members CARP/Ankrd1 and Ankrd2/Arpp. Their role in pathological conditions will be discussed, with particular emphasis on their expression in tumors and potential use as diagnostic and prognostic markers.

Structure and function of CARP/Ankrd1 and Ankrd2/Arpp

CARP/Ankrd1 has been independently identified by several groups as a cytokine-inducible transcriptional regulator, a protein interacting with transcriptional factor YB-1, and a cardiac doxorubicin-responsive protein (3–5). In normal tissues, it is highly expressed in cardiac muscle and detectable in skeletal muscles (4). It is an early differentiation marker during cardiogenesis with a high expression level in developing heart (4, 5). CARP/Ankrd1 has been found to be induced in the hypertrophy, during cardiac ventricle overload induced by banding of the descending aorta (6) and in skeletal muscle denervated by transection of the sciatic nerve (7, 8). In skeletal muscle, CARP/Ankrd1 is up-regulated in response to a variety of mechanical stresses, including acute resistance exercise (9) and work overload hypertrophy (10).

From a functional point of view, CARP/Ankrd1 is a transcription co-inhibitor that acts downstream of the homeobox gene Nkx2.5 pathway in cardiomyocytes (4), and its overexpression suppresses toponin C and atrial natriuretic factor expression (5). Its interaction with the transcription factor YB1 promotes inhibition of the ventricular specific myosin light chain 2v (MLC-2v) (4). In vascular smooth muscle cells, increased CARP/Ankrd1 expression is associated with up-regulation of the $p21^{WAF1/CIP1}$, a universal inhibitor of the cell cycle (11). Laure and coauthors have demonstrated that the p21 gene expression profile parallels that of CARP/Ankrd1 in muscular dystrophy and denervation-induced atrophy models (12). We have demonstrated that CARP/Ankrd1 can act as a transcriptional co-activator, moderately up-regulating p53 activity of the $p21^{WAF1/CIP1}$ promoter. It interacts with tumor suppressor protein p53 and has stimulatory activity on p53 regulatory function. Taken together, these findings suggest that CARP/Ankrd1 coordinates the expression of genes involved in cell structure and proliferation, and could play a role in muscle remodeling.

Apart from the interaction with transcription factors, CARP/Ankrd1 binds the sarcomeric protein titin (1) and cardiac calsequestrin-2, CASQ2 (13). These interaction are mediated, at least partially, by the binding sites localized within the ankyrin repeats and coiled-coil domain. CARP/Ankrd1 can also interact with the sarcomeric proteins myopalladin (2), desmin (14), and muscle-specific RING finger proteins MuRF1/MuRF2 (15) indicating its structural role.

The Ankyrin repeat domain 2 (*Ankrd2)* gene was first detected as a novel transcript up-regulated in mouse muscle after 7 days of stretch (16). It is more expressed in skeletal than cardiac muscle, preferentially in type 1 fibers (8, 17). Its expression in skeletal muscle is increased in denervated muscles (8) and both CARP/Ankrd1 and Ankrd2/Arpp are induced in muscles after eccentric contractions (18, 19). During the differentiation of skeletal muscle cells in culture, Ankrd2/Arpp is expressed both in the nucleus and cytoplasm of myoblasts and mainly in the cytoplasm upon differentiation (17). Although it has been reported to be localized mainly in the I-band of sarcomere, it can interact, both *in vitro* and *in vivo*, with nuclear transcription factors, including p53, promyelocytic leukemia protein (PML) and YB-1, and may regulate their transcriptional activity (20). Apart from transcription factors, Ankrd2/Arpp interacts with the sarcomeric proteins telethonin (20) and titin (1), suggesting that it may translocate from the I band to the nucleus and participate in signaling pathways activated in response to muscle stress. It has already

been documented that Ankrd2/Arpp accumulates in the nuclei of myofibers located adjacent to severely damaged myofibers (21).

The precise roles of CARP/Ankrd1 and Ankrd2/Arpp are not fully elucidated. The diverse range of their binding partners, including transcription factors (YB-1, PML, p53), myofibrillar (titin, myopalladin, telethonin), intermediate filament (desmin), calcium-handling (CASQ2), and ubiquitin ligase (MuRF1/MuRF2) proteins, suggests the pleiotropic and integrative functions of both proteins in striated muscle. Protein–protein interactions with functionally diverse proteins and the dual nuclear-cytoplasmic localization of CARP/Ankrd1 and Ankrd2/Arpp point to their structural and regulatory functions as components of a titin associated stretch-sensing complex in the myofibril and as co-factors of transcription in the nucleus.

CARP/Ankrd1 and Ankrd2/Arpp in disease

As muscle specific proteins, with both structural and regulatory roles, their expression is altered in cardiac and skeletal muscle disorders. Interestingly, the emerging role of CARP/Ankrd1 and Ankrd2/Arpp in tumor diagnosis and prognosis has also been demonstrated.

CARP/Ankrd1 expression is rapidly induced in cardiomyocytes at heart failure, indicating its possible involvement in pathological remodeling of the myocardium. Its expression is increased in patients with left ventricular dilated and ischemic cardiomyopathies (22), and it has been identified as a candidate gene with a role in congenital heart disease (23). Recently, several missense mutations in CARP/Ankrd1 gene were identified in DCM (dilated cardiomyopathy) and HCM (hypertrophic cardiomyopathy) patients, causing impaired protein–protein interactions and altered gene expression in response to mechanical stretch (24–26).

In skeletal muscle, CARP/Ankrd1 and Ankrd2/Arpp have altered expression patterns in patients suffering from muscular dystrophies (MD), congenital myopathy (CM) and spinal muscular atrophy (SMA). It was found that CARP/Ankrd1 is differentially induced in DMD and congenital muscular dystrophy (CMD). In DMD, CARP/Ankrd1 tends to be expressed in small myofibers, morphologically similar to regenerating myofibers that express embryonic myosin heavy chains (MHC). The expression pattern similar to that of experimentally induced regenerating muscle suggests that it may be induced during regeneration after muscle necrosis in DMD. On the other hand, in CMD patients CARP/Ankrd1 expression was not limited to the regenerating myofibers, but was observed in myofibers coexpressing either embryonic or mature-type MHC (27). CARP/Ankrd1 was also preferentially induced in atrophic myofibers in muscles of CM and SMA patients. It was selectively expressed in severely atrophic myofibers in SMA patients, indicating that CARP/Ankrd1 expression may reflect the status of muscle atrophy. In CM, the expression patterns of CARP/Ankrd1 were distinct among the subtypes. In nemaline myopathy, it was found preferentially expressed in severely damaged myofibers, but was not detected in central core disease (28).

Ankrd2/Arpp has been found to be down-regulated in patients with MD, while it was up-regulated in atrophic or damaged myofibers in patients with CM. In SMA patients Ankrd2/Arpp was induced in hypertrophic myofibers and Ankrd2/ Arpp-positive myofibers were arranged in groups as a result of the process of denervation (29).

CARP/Ankrd1 and Ankrd2/Arpp in tumors

There are a few reports of CARP/Ankrd1 and Ankrd2/Arpp expression in neoplasia and the existing knowledge on their role in cancer is limited. It has been reported that both proteins are expressed in rhabdomyosarcomas. Ankrd2/Arpp was detected in renal oncocytomas, while the CARP/Ankrd1 expression was observed in ovarian and breast cancer cell lines, as well as in the majority of serous ovarian adenocarcinomas.

Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is a malignant soft tissue tumor accounting for 20% of all sarcomas in children and adolescents. Tumor cells resemble primitive skeletal muscle cells and usually show a striated muscle-like differentiation. The diagnosis of RMS is based on the detection of specific features of skeletal muscle phenotypes such as cross-striation. Immunohistochemical analysis of muscle specific markers is also used in cases when cross-striation is absent. Muscle specific actin, desmin and myoglobin, as well as myogenic transcription factors myogenin and MyoD, have been used for diagnosis of RMS (30–36). The advantage of myogenin and MyoD is that they are expressed in the least differentiated tumors, therefore they can be used as tumor markers for early diagnosis. All these markers vary in their sensitivity and specificity, and the search for a more suitable disease marker is still in progress. Potential candidates could be MARP family members CARP/Ankrd1 and Ankrd2/Arpp due to the presence of positive cells for both proteins in all tested RMS cases (37). In order to evaluate Ankrd2/Arpp as a potential tumor marker for the differential diagnosis of RMS, Ishiguro and coauthors (38) have studied its expression among various soft tissue sarcomas. Formalin-fixed and paraffin-embedded tissue samples of

37 RMS, 88 non-RMS sarcomas and 38 carcinomas were immunohistochemically analyzed for Ankrd2/Arpp expression. It was detected in 89.2% of RMS cases, and in all samples when Western blot was used as the detection method. In contrast, the expression of Ankrd2/Arpp in non-RMS and other carcinomas was very weak.

Recently, an immunohistochemical analysis of CARP/Ankrd1 expression was also performed using 159 malignant tumors. Its cytoplasmic immunoreactivity was observed in 85% of RMS cases and in only 4% of non-RMSs (39). These results indicate that CARP/Ankrd1 and Ankrd2/Arpp may have a potential use in the diagnostics of RMS.

Renal oncocytomas

Renal oncocytoma is a type of epithelial neoplasm, considered as a benign tumor originating from the renal collecting duct cells. Oncocytes are large well-differentiated neoplastic cells with intensely eosinophilic granular cytoplasm due to the large number of mitochondria (40–42). Ankrd2/Arpp, preferentially expressed in striated muscle, is also found in the kidney, as demonstrated by RT PCR and Western blot analysis of different human tissues (17). In the normal kidney, Ankrd2/Arpp is localized in mitochondria and nuclei, in part of the distal renal tubule, in both the renal cortex and medulla. Immunohistochemical analysis of 100 renal tumors (14 oncocytomas and 86 renal cell carcinomas (RCC)) revealed high expression of Ankrd2/Arpp in oncocytoma (12 of the 14 cases, 85.7%). It was not detected in 11 analysed cases of malignant chromophobe RCCs, suggesting its potential use in differential diagnosis between benign and malign renal tumors (43).

Ovarian cancer

Platinum-based chemotherapy is used for standard treatment of epithelial ovarian cancer. These agents are initially effective, but usually the disease relapses after a variable interval. So far, clinical prediction of platinum sensitivity and strategies to overcome platinum resistance are not possible. Scurr and colaborators (44) identified a panel of genes with altered expression associated with high sensitivity to cisplantin. A significant correlation was observed between the CARP/Ankrd1 level and platinum response. It was found that CARP/Ankrd1 was specifically decreased in the platinum sensitive cell lines. Analysis of 71 serious ovarian cancer patients has revealed that patients with a moderate to high level of CARP/Ankrd1 had a worse outcome compared with patients whose tumors had low CARP/Ankrd1 expression. It was demonstrated that decreasing CARP/Ankrd1 expression correlates with sensitivity of ovarian cells to cisplantin and its level was negatively correlated with cisplantin sensitivity in a panel of human cancer cell lines.

MARPs as potential diagnostic and prognostic tumor markers

The expression of MARPs in RMS was studied in order to evaluate them as potential diagnostic markers. The markers of early myogenic differentiation used routinely in clinical practice (such as myogenin and MyoD) are not always reliable. Ankrd2/Arpp expressing cells did not always co-express other muscle specific markers such as desmin, fast MHC or muscle actin, indicating that Ankrd2/Arpp can be detected in RMS cells that are undetectable by other existing tumor markers (37). In comparison with myogenic markers, the sensitivity of Ankrd2/Arpp was higher than that of myoglobin (89.2% and 59.6% respectively) and comparable with myogenin, MyoD, muscle-specific actin and desmin sensitivity (38). The specificity and sensitivity of CARP/Ankrd1 were similar to those observed for other myogenic markers (39). Taken together, the results of various studies indicate that CARP/Ankrd1 and Ankrd2/Arpp clearly have higher expression in RMS than in non-RMS tumors. They are sensitive and specific markers for RMS and might be useful for the differential diagnosis of RMS.

The differential diagnosis between benign renal oncocytoma and malignant chromophobic RCC is difficult because this variant of the RCC tumors is morphologically similar to renal oncocytoma and a precise diagnosis has prognostic significance. Existing markers, including vinculin, paxillin, SHP2, parvalbumin and c-kit are used to distinguish these two types of tumors. Caveolin is reported to be expressed in 91.7% of oncocytomas, but it was also detected in about 20% of RCC variants. Thus, caveolin on its own is not sufficient to separate the two tumors based on its expression profile. CD10, a marker for the proximal renal tubule was expressed in every subtype of renal tumor except for chromophobe RCC. A marker for distal renal tubule, EMA (epithelial membrane antigen) was highly expressed in oncocytomas, as well as in other subtypes. Since Ankrd2/Arpp is selectively expressed in oncocytomas but rarely in other types of renal tumor, its potential use as a specific marker for distinguishing oncocytomas from chromophobe RCC is proposed (43).

Recently, it has been suggested that CARP/Ankrd1 has a role in determining cellular response to platinum-based chemotherapy in ovarian cancer treatment (44). Decreasing CARP/Ankrd1 expression or function could be a potential strategy to sensitize tumors to platinum-based drugs and avoid disease relapse. Thus, it represents a novel target for pharmacological inhibition in order to sensitize tumors to platins and increase its clinical effectiveness.

CARP/Ankrd1 and Ankrd2/Arpp in molecular mechanisms of tumorigenesis

Several findings indicate a potential molecular mechanism by which CARP/Ankrd1 and Ankrd2/Arpp might be involved in the process of tumorigenesis. A functional interaction between the tumor suppressor protein p53 and both CARP/Ankrd1 and Ankrd2/Arpp has been demonstrated (20). They interact with and enhance p53 trans-activation ability. Furthermore, p53 up-regulates CARP/Ankrd1 and Ankrd2/Arpp promoters, suggesting its role in the regulation of their expression. The complex relationship of CARP/Ankrd1 and Ankrd2/Arpp with p53 causing the fine tuning of p53 activity implicates that an alteration in their interdependancy could lead to pathogenicity, such as the process of cancerogenesis. Cancer is usually associated with aberrant cell cycle progression and defective apoptosis induction due to the activation of proto-oncogenes and/or inactivation of tumor suppressor genes (45). p53 is a well-established and frequently mutated tumor suppressor in human cancer. Since its discovery as a tumor suppressor (46), p53 has been the hot spot gene for studying the mechanisms of tumor formation (47–49). The p53 protein acts biochemically as a transcription factor and biologically as a powerful tumor suppressor. Under normal, unstressed conditions, the p53 protein remains undetectable due to its short half-life. The p53 instability is primarily controlled by its negative regulator, an E3 ubiquitin ligase Mdm2 (murine double minute), which targets p53 for proteasome-mediated degradation (50, 51). p53 responds to a wide variety of cellular stresses including genotoxic damages, oncogene activation, hypoxia and mechanical stretch (52–54). It is capable of adjusting the biological response to the nature of the activating signal; it can trigger apoptosis when cells are confronted with severe, presumably irreparable damage, while imposing a growth arrest when the damage is mild, thus enabling the cell to fix the damage and resume normal life (55, 56). p53 is inactivated by point mutations in more than 50% of human cancers (http://www.iarc.fr/p53) with a majority of mutations occurring in the DNA binding domain, which either changes wt p53 conformation or abolishes its interaction with DNA (57). Furthermore, in cancer carrying a wt p53, p53 is often nonfunctional as a result of either being degraded by overexpressed Mdm2 (50, 51) or being excluded from the nucleus where p53 acts as a transcriptional factor (58–60). We defined the tumor suppresor protein p53 as a common downstream target for both CARP/Ankrd1 and Ankrd2/Arpp proteins. They bind p53 and enhance the p53-mediated up-regulation of the cell cycle inhibitor $p21^{WAF1/CIP1}$ promoter (20). In turn, p53 enhances CARP/Ankrd1 and Ankrd2/Arpp promoter activity, implicating a role of p53 in the regulation of its effectors (*Figure 2*). Preventing an association between MARPs and p53 could be a potential mechanism by which CARP/Ankrd1 and Ankrd2/Arpp might play a role in tumorigenesis.

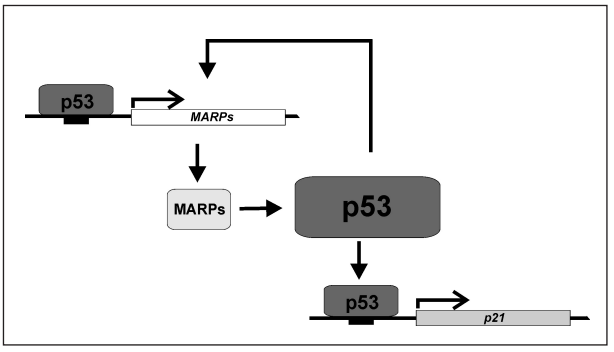

Figure 2 Schematic representation of multilevel interaction between MARP family members CARP/Ankrd1 and Ankrd2/Arpp and tumor suppressor p53.

CARP/Ankrd1 or Ankrd2/Arpp could also participate in other pathways involved in tumorigenesis such as transforming growth factor-β (TGF-β) and Wnt signaling pathways. Alterations in these pathways are associated with several pathological conditions, including cancer. Cells costimulated with TGF-β and Wnt3a displayed an expression profile distinct from the one observed with single ligand treatments, as demonstrated by a microarray-based aproach. The induction of both pathways generates the expression of a unique set of genes, and CARP/Ankrd1 is one of them (61). Thus, CARP/Ankrd1 and Ankrd2/Arpp may be cooperative targets displaying altered expression and/or function in tumors, promoting their development.

In conclusion, we can speculate that CARP/Ankrd1 and Ankrd2/Arpp are good candidates for diagnostic use, being specifically expressed in certain tumors. This observation is further corroborated with their activity in the fine modulation of the tumor suppressor protein p53 function and a role in the pathways known to participate in the mechanisms of tumorigenesis. All available data on CARP/Ankrd1 and Ankrd2/Arpp expression in tumors have been obtained from a rather small number of patients and in order to further evaluate them as tumor diagnostic and prognostic markers, more studies with a larger number of patients from different populations are required.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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