Endogenous Vaults and Bioengineered Vault Nanoparticles for Treatment of Glioblastomas

Implications for Future Targeted Therapies

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KEYWORDS

• Brain tumor • Immunotherapy • Nanoparticle • Targeted therapy • Vault

KEY POINTS

- Endogenous vaults are ribonucleoproteins expressed throughout various cell types and across numerous species.
- The vault has been hypothesized to play a role in cellular transport implicated in innate immunity, multidrug resistance, and intracellular signaling.
- Gangliogliomas, schwannomas, meningiomas, neurofibromas, astrocytomas, and gliomas have all been reported to exhibit high levels of major vault protein (MVP), which constitutes approximately 70% of the overall mass of endogenous vaults.
- In vitro culture of dendritic cells with antibodies against MVP demonstrates decreased dendritic cell functioning, particularly a reduction in their ability to induce antigen-specific T cell proliferation, indicating that MVP may play a critical role in dendritic cell activation and cellular immunity.
- Bioengineered vault nanoparticles seem to be ideally suited for use as nanocapsules in the delivery of various therapeutic agents and immunogenic proteins, representing a promising prospect for CNS tumor immunotherapy.

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INTRODUCTION

Endogenous vaults are the largest ribonucleoproteins expressed in numerous higher organism species and cell types. Their precise role and function, however, remain largely uncharacterized. The 4 major components of endogenous vaults are major vault protein (MVP), vault poly (ADP) ribose polymerase (vPARP), telomerase-associated protein (TEP1), and untranslated vault RNA (vRNA) molecules. MVP, also described as human lung resistance protein (LRP), has been investigated for its expression in numerous cancers and its possible role in chemoresistance. This article provides an overview of endogenous vaults and the work on bioengineered vault nanoparticles in order to better elucidate the potential role that these complexes may play in a targeted therapy for the central nervous system (CNS). It will focus, in particular, on primary human brain cancers such as malignant gliomas. Lastly, the authors hope to highlight the implications that current research holds in potentially utilizing bioengineered vault nanoparticles for future targeted therapies against human glioblastoma.

VAULT NANOPARTICLES: AN OVERVIEW

Vaults were first characterized by Kedersha and Rome in 1986.[1,2] By using a negative stain for electron microscopy (EM) rather than the positive-staining heavy metal salts that detect nucleic acid and membrane components, Kedersha and Rome were able to visualize the presence of protein-rich nanoparticles with a structural morphology similar to vaulted ceilings in cathedrals.[1,2] Vaults have since been characterized as barrel-like ribonucleoproteins with a mass of approximately 12.9 MDa and dimensions of 420 $\times$ 420 $\times$ 750 Å. This enormous size classifies vault nanoparticles as among the largest ribonucleoproteins (RNPs) ever characterized.[1,3–7]

The macrostructure of endogenous vaults have been investigated in order to elucidate key insights into their cellular function. The 4 main components of the vault nanoparticle are the structural 100 kDa MVP, the enzymatic 193 kDa ADPvPARP, the RNA-binding 240 kDa TEP1, and vRNA.[8–10] MVP and vRNAs constitute approximately 70% and 5% of the overall mass of endogenous vaults, respectively.[4,11] In each cell, there may be between 10,000 and 10,000,000 of these endogenous barrel-like complexes.[12,13] Vaults localize predominately to the cytoplasm of the cell, but they occasionally can be found in the nucleus, congregating around nucleoli, on the outside of the nuclear envelope, and at the nuclear pore complexes (NPCs), potentially mediating exchange across the nuclear membrane.[14–16] In the cellular cytoplasm, endogenous vaults can be associated with cytoskeletal elements such as actin stress fibers or microtubules.[14,17,18]

Kedersha and colleagues initially utilized quantitative scanning transmission EM to describe bioengineered vault nanoparticles as dimers, with each half resembling a barrel-like structure that consists of 8 rectangular petals that open into a flowerlike structure (**Fig. 1**). Each petal was hypothesized to consist of 6 molecules of MVP.[4] Cryo-EM and single-particle reconstruction indicate that the vault nanoparticle is a hollow, barrel-like structure with an approximate volume of 5×10^7 Å^3.[19] The protein shell of the vault has an invaginated waist with 2 caps protruding on either end of the vault.[6,19–22] Although some evidence has confirmed Kedersha's initial hypothesis of an 8-fold dihedral symmetry, more recent investigations suggest a potential 39-fold dihedral axis or 42-fold rotational symmetry.[20,23,24] In either instance, the bioengineered vault nanoparticle seems to consist of a barrel-like structure with 78 to 96 MVP molecules arranged pole-to-pole, and 39 to 48 copies of MVP forming each half-vault.[6,20,21] For endogenous vaults, each vault has been predicted to contain 1 or more copies of TEP1 and approximately 4 copies of VPARP; additionally, the vRNA also appears to localize to the ends of the vault caps, where it associates with TEP1.[6,10,25]

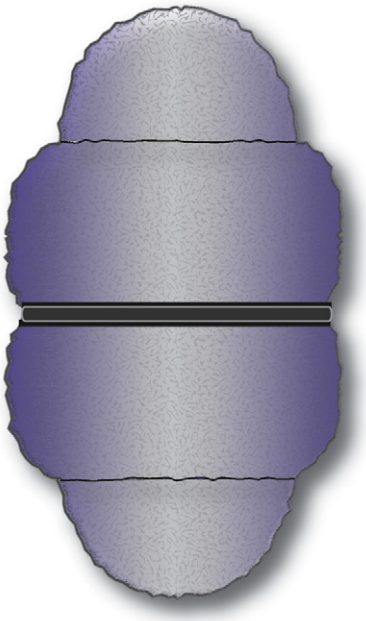

Fig. 1. A schematic rendering of the bioengineered vault nanoparticle, which may encapsulate a therapeutic agent or immunogenic antigen.

One of the most interesting findings of endogenous vault investigations has been their reported upregulation in certain types of cancers. For example, expression upregulation has been reported in breast tumors,[26,27] nonsmall cell lung cancer (NSCLC),[28–30] and other malignancies.[31–49] Several researchers have also reported upregulation of MVP in several brain tumors. Gangliogliomas, schwannomas, meningiomas, neurofibromas, astrocytomas, and gliomas have all been reported to exhibit high levels of the MVP protein.[50–54]

Functionally, the endogenous vault barrel-like structure has been hypothesized to have a possible role in the cellular transport implicated in innate immunity, multidrug resistance (MDR), and intracellular signaling.[55] Most recently, vaults have been studied as possible vectors for therapeutic delivery. Here, the authors will identify and discuss the endogenous vault components and highlight their expression in the CNS and CNS tumors. They will also discuss future implications for targeted therapeutics and the potential role of bioengineered vault nanoparticles for the induction of immune responses and their prospective utilization as a novel method of immunotherapy.

MVP: THE MVP SUBUNIT

The human MVP gene localizes to chromosome 16p11.2.[56–58] The MVP protein also structurally forms the outer shell of the bioengineered vault nanoparticle.[19] Several interesting structural domains have been described within MVP. First, the C-terminus of MVP contains a coiled-coil protein structure that is formed by a long α-helical domain.[59] This C-terminal appears to localize to the vault end cap, and it is hypothesized that these coiled-coil interactions allow MVP particles to interact with one another, providing a likely mechanism for how vaultlike complexes were demonstrated in vault-less Sf9 insect cells after ectopic rat MVP was expressed in these cells.[21,60] When the coiled-coil domain is partially deleted in a yeast 2-hybrid system, this interaction does not appear possible.[59] Secondly, the N terminus of MVP consists of 7 repeats of approximately 55 amino acids. This terminus localizes to the vault's equator, where it forms the sidewalls via noncovalent interactions between particle halves. In addition, part of the N terminus also protrudes interiorly from this equator waistline.[6,20,21,61]

MVP, the major component of bioengineered vault nanoparticles, has also been implicated in innate immunity. While dendritic cell function is still normal in mice that lack MVP,[62] in vitro culture of dendritic cells with antibodies against MVP demonstrate decreased dendritic cell functioning, particularly a reduction in their ability to induce antigen-specific T-cell proliferation.[63] These findings suggest that MVP may play a critical role in innate immunity and dendritic cell activation, providing a key component for the utilization of vault nanoparticles for an immunotherapeutic approach.

vPARP: THE vPARP SUBUNIT

The vPARP gene, located on chromosome 13q11, encodes a protein with 1724 amino acids.[9,64] The enzymatically active PARP domain was first described after an experiment demonstrated that vPARP is able to catalyze the ADP ribosylation of both itself and MVP.[9] ADP ribosylation is most often associated with a post-translational modification of histones and other nuclear proteins that occur principally in response to DNA damage by PARP1. Enzymes with a PARP domain are able to transfer ADP-ribose groups to aspartic and glutamic residues. These altered substrates interact differently with DNA, creating a delay in DNA replication that gives cells enough time to recruit repair enzymes to the site of the DNA damage.[65–67] At least 7 proteins with PARP activity have been described, with PARP1 being the best characterized in this family. These family members, however, are not homologous outside of their catalytic domains.[68,69]

The archetypal PARP1 binds to single- or double-strand DNA breaks in the nucleus and appears to play a role in the base excision pathway.[68,70] Interestingly, vPARP shares a 28% sequence homology with PARP1 but does not appear to be activated in response to DNA damage.[9] This is further suggested by experiments with knockout mice in which a (-/-) vPARP genotype demonstrated neither chromosomal instability nor endogenous vault disturbances.[71] However, a significant increase in carcinogen-induced tumors has been reported in vPARP-deficient mice and may indicate a potential pathway for vPARP in chemically-induced neoplasia.[72]

TEP1: THE TEP1 SUBUNIT

TEP1, the other endogenous vault associated protein, was found to be identical to the previously described mammalian telomerase-associated component TEP1.[10,73] The human TEP1 gene maps to chromosome 14q11.2. The TEP1 protein is comprised of 2629 amino acids and contains a number of interesting domains: an amino–terminal repeat domain, an RNA binding domain, and an adenosine triphosphate (ATP)/guanosine triphosphate binding domain.[73,74] The repeats at

the N terminus do not have a known function, but have been hypothesized to serve as binding sites for vPARP.[6] The RNA binding domain appears to be necessary for interaction with both vRNA and telomerase RNA.[75–77]

Endogenous vaults have been isolated from TEP1 knockout mice that appear normal; however, closer examination by 3-dimensional reconstruction demonstrates reduced density in the vault cap, where TEP1 appears to localize. Furthermore, the stable association between vRNA and the vault complex is completely disrupted in these knockouts. Both the half-life of vRNA and its expression in these tissues decrease. Thus, it appears that TEP1 seems to play a key role in vRNA stability and its binding to the vault complex.[25]

UNTRANSLATED RNA

The human vRNA genes are a triple-repeat structure on chromosome 5q33.1.[8,10,12,25,78,79] The vRNAs are transcribed by RNA polymerase 3,[80] but their expression in each cell seems to differ by organ. Data from northern analysis suggests that one of the lowest levels of vRNA expression occurs in the brain.[8]

Concentrated at the ends of vault caps, vRNAs appear to interact with structural endogenous vault proteins, but it does not appear that they are required to maintain the structural integrity of the bioengineered vault nanoparticle. vRNA loss by ribonuclease digestion fails to cause any detectable conformational change of the bioengineered vault nanoparticle complex. This suggests that vRNAs do not play a critical structural role in bioengineering of vault nanoparticles.[4,6,81]

VAULTS AND THE CNS

While endogenous vaults are present in all cell types, they appear to be differentially expressed depending on the cell of origin. The highest levels of MVP/LRP are found in macrophages, keratinocytes, kidney tubules, and epithelial cells of the bronchus and digestive tract.[13,50,64,82] Brain tissue, however, appears to only have low levels of MVP/LRP.[50,52,53] In rat brains, the highest expression of MVP is found in microglia during embryonic development, but in the adult animal, MVP is evident in the amoeboid microglia and choroid plexus. The motile character of these structures may explain their association with endogenous vaults, given the potential of endogenous vaults to interact with cytoskeletal elements.[18,83]

Upregulation of MVP/LRP, however, has been reported in a wide range of brain tumors.[50–54,84] Conversely, medulloblastomas and neuroblastomas have been reported to demonstrate low levels of MVP.[51] Increased levels of MVP/LRP have also been found in tumor vessels, in dorsal root ganglion tissue after nerve ligation, and in macrophages after brain infarction or acute contusion injury.[52,53,85] Berger and colleagues, utilizing both mRNA and protein assays, have reported high levels of MVP in astrocytomas, meningiomas, and gliomas. With cytotoxicity analysis of astrocytomas, this study also reported that MVP expression was significantly correlated with resistance to adriamycin, daunomycin, etoposide, and cisplatin.[51]

To further characterize the expression of MVP in primary tumors of the CNS, Sasaki and colleagues performed immunohistochemistry on 69 archival CNS tumors and demonstrated that 56 of the samples (81.2%) expressed LRP. Specifically, the protein was found in specimens of neurofibroma, schwannoma, meningioma, oligoastrocytoma, ependymoma, oligodendroglioma, and astrocytoma. Interestingly, neither tumor grade nor invasion appeared to be correlated with the presence of MVP.[84,86]

Focusing on primary and secondary glioblastomas, Tews and colleagues reported consistent upregulation of MVP. Moreover, 78% of World Health Organization (WHO) grade II precursor astrocytomas and all WHO grade III tumors exhibited MVP expression. Because none of these tissues had been subjected to previous chemotherapy, increased MVP expression was likely due to the inherent upregulated expression of MVP in glioma cells, which differs from the low levels of MVP in normal glial cells.[52]

POTENTIAL USES FOR FUTURE THERAPIES

Due to the large internal volume and simple structure of bioengineered vault nanoparticles, they seem to be ideally suited for use as nanocapsules for delivery of therapeutic agents. Several successful encapsulations have been described. In 2005, Kickhoefer and colleagues were able to engineer a vault nanoparticle complex that sequestered 2 proteins and remained viable in living cells.[61] Goldsmith and colleagues were able to demonstrate the capability to load the vault nanoparticle interior,[87] while Ng and colleagues were also able to effectively use the bioengineered vault cage to enclose a semiconducting polymer.[88]

To analyze their potential therapeutic potential, Esfandiary and colleagues investigated the bioengineered vault nanoparticle structural changes in response to various pH and temperature conditions. This analysis found that bioengineered vault nanoparticles remained the most stable below

40°C and that the flowerlike petal structures open at an acidic pH. This opening could serve as a potential mechanism to release therapeutic contents from inside the bioengineered vault nanoparticle.[89] Investigating a different potential mechanism of delivery, Lai and colleagues incorporated the membrane lytic domain of adenovirus protein VI (pVI) into vault interiors, where its activity was maintained. After being ingested by murine macrophages, these novel bioengineered vault nanoparticle complexes aided in the delivery of a soluble ribotoxin and a cDNA plasmid.[90]

Recently, Kickhoefer and colleagues have constructed vault nanoparticles to specifically target the epidermal growth factor receptor (EGFR) by tagging the C terminus of MVP with wither epidermal growth factor or a monoclonal antibody to EGFR.[91] Champion and colleagues recently engineered vault nanoparticles to deliver the major outer membrane protein of *Chlamydia muridarum*. In mice, the application of these bioengineered vault nanoparticles induced mucosal immunity without inducing harmful inflammation. This novel vaccine, utilizing bioengineered vault nanoparticles delivered intranasally, represents a significant advance in the ability to induce an effective cellular immune response utilizing vault nanoparticles.[92]

SUMMARY

Further studies are needed to elucidate the mechanisms of endogenous vault function and gene expression. These advances may enable the development of targeted therapies that may prevent cancer cells from acquiring MVP-related drug resistance. The use of bioengineered vault nanoparticles as delivery vectors for therapeutic agents and immunogenic proteins may be a promising utilization for vault nanoparticles as a potential method of brain tumor therapy.

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