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Gene therapy for Heart Failure: New Perspectives

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Abstract

Purpose of review.—The current knowledge of pathophysiological and molecular mechanisms responsible for the genesis and development of heart failure (HF) is absolutely vast. Nonetheless, the hiatus between experimental findings and therapeutic options remains too deep, while the available pharmacological treatments are mostly seasoned and display limited efficacy. The necessity to identify new, non-pharmacological strategies to target molecular alterations led investigators, already many years ago, to propose gene therapy for HF. Here, we will review some of the strategies proposed over the past years to target major pathogenic mechanisms/factors responsible for severe cardiac injury developing into HF and will provide arguments in favor of the necessity to keep alive research on this topic.

Recent findings.—After decades of preclinical research and phases of enthusiasm and disappointment, clinical trials were finally launched in recent years. The first one to reach phase II and testing gene delivery of sarcoendoplasmic reticulum calcium ATPase did not yield encouraging results, however other trials are ongoing, more efficient viral vectors are being developed and promising new potential targets have been identified. For instance, recent research is focused on gene repair, *in vivo*, to treat heritable forms of HF, while strong experimental evidence indicates that specific microRNAs can be delivered to post-ischemic hearts to induce regeneration, a result that was previously thought possible only by using stem cell therapy.

Summary.—Gene therapy for HF is aging, but exciting perspectives are still very open.

Gene therapy: still an appealing option for the treatment of heart failure

Thanks to the joint effort of numerous investigators (1), the pharmacological treatment of heart failure (HF) is reaching a plateau that, save for possible major breakthroughs, will be hardly overcome in the near future. In fact, after decades of stalemate, only two drugs, namely sacubitril/valsartan and ivabradine, have been added to the seasoned pharmaceutical arsenal available to cardiologists for the treatment of this syndrome. (2) Unfortunately, severe HF is still characterized by very negative prognosis (1) and the therapeutic perspectives do not seem glaring, despite the cornucopia of studies that yielded a deep

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knowledge of cellular and molecular pathogenic mechanisms underlying this syndrome. The difficulty of pharmacologically targeting receptors and intracellular pathways involved in the pathogenesis of HF led investigators, many years ago (3), to propose cardiac gene therapy as an alternative therapeutic approach. The idea was simple and revolutionary at the same time: cell-directed delivery of exogenous genes (transgenes) would produce “curative” proteins able to compensate for pathological downregulations or to counteract detrimental molecular processes. Investigators in the field soon realized that the careful choice of proper targets and the availability of efficient gene carriers were critical requisites for the success of this therapeutic approach.

The history of cardiac gene therapy began with a seminal study published by Dr. Leiden’s group in 1990 (4). Those authors transfected rat cardiomyocytes, *in vivo*, by injecting plasmid DNA containing the β -galactosidase gene directly in left ventricular wall. β -galactosidase activity was then found in myocardium for up to 4 weeks. The 3 decades following that milestone report witnessed a real hype for HF gene therapy, which, after formidable efforts and persistence of courageous investigators, culminated with the first clinical trials in recent years.

HF is a complex, multifactorial syndrome. That is why authors have tested very diverse targets ranging from enzymes to structural proteins and cytoprotective factors. Here, we will review some of the strategies proposed over the past years to target major pathogenic mechanisms/factors responsible for severe cardiac injury developing into HF, and will provide arguments in favor of the necessity to keep alive research on this topic. We refer the reader to other reviews covering in detail the principles of gene therapy and its numerous applications in the cardiovascular field. (5–8)

Rescuing SERCA2a function

Sarcoendoplasmic reticulum calcium ATPase (SERCA2a) is a protein pump critically important for cytosolic Ca^{2+} homeostasis in cardiac myocytes. It works in coordination with ryanodine channels and $\text{Na}^+/\text{Ca}^{2+}$ exchangers, removing Ca^{2+} from the cytosol during the repolarization/relaxation phase. SERCA2a activity is modulated by the integral membrane protein phospholamban (PLN) via inhibitory phosphorylation. In turn, PLN is inhibited by phosphorylation with consequent release of the brake on SERCA2a and enhanced Ca^{2+} uptake by sarcoplasmic reticulum (9). There is evidence that SERCA2a is downregulated in failing hearts, leading to reduced Ca^{2+} re-uptake by the sarcoplasmic reticulum during diastole, hence impaired relaxation, higher energy requirements and predisposition to arrhythmias. Moreover, Ca^{2+} depletion in the sarcoplasmic reticulum has serious repercussions on the excitation/contraction coupling and therefore cardiac force generation (6, 10–11). Experimental models proved the concept that PLN gene deletion or forced expression of a constitutively phosphorylated form of PLN result in improved heart function. (12–14) But the hypothesis that ultimately led to a clinical trial was that SERCA2a gene transfer to cardiac cells might restore the physiological expression of this molecular pump and increase cardiac contractility in HF (15). After successful tests in isolated human cardiomyocytes (15), murine (16) and porcine models (17), this strategy was finally translated to the clinics, with the initiation in 2012 of CUPID (Calcium Up-Regulation by

Percutaneous Administration of Gene Therapy in Cardiac Disease), the first clinical trial of cardiac gene therapy for HF. Replication defective adeno-associated virus of serotype 1 (AAV1) was the vector chosen to carry SERCA2a transgenes. While the initial phases demonstrated safety and some beneficial effects (18), the final Phase 2b CUPID was unsuccessful. It involved 250 patients receiving intracoronary AAV1/SERCA2a or placebo. The treatment with AAV1/SERCA2a failed to prolong the time to the first terminal event. (19–20) Compared to placebo, treatment with AAV1/SERCA2a had no significant effect on any endpoints, including NYHA Functional class, 6-minute walk test distance or NT-proBNP levels (20).

Despite its failure, CUPID2b finally made gene therapy “palpable” in clinical cardiology after decades of preclinical research. The potential factors that caused the negative outcome, rather than discouraging the reiteration of similar attempts, should be carefully analyzed and spur further research and optimization. Possible problems relied in the selection of the patient population or/and of the endpoints, but also in the insufficient delivery or intracellular transfer of SERCA2a gene. In the CUPID2b trial, myocardial uptake of AAV vectors was ~1000 times lower than in animal models (20). Therefore, attempts should be made to improve transduction efficiency. For instance, the anterograde coronary delivery could perhaps be replaced by retrograde perfusion to prolong the transit time of the vector in capillaries. Another major issue is the low transduction efficiency of the AAV itself. Increasing the vector dose is not necessarily the right solution. Several teams tried to reengineer AAVs. For instance, some authors exploited the mechanism of “directed evolution”, a method of in vitro evolution followed by in vivo selection of AAV capsids targeting a certain organ, to generate an AAV mutant with cardiac-specific tropism (21). They obtained a capsid gene clone named M41 that displayed tenfold higher transduction efficiency in cardiac tissue compared with the liver. Another team of investigators transduced large animal hearts using reengineered AAVs, named BNP116, to deliver Protein Phosphatase Inhibitor-1 Gene (20). BNP116 is a synthetic AAV vector developed by [site-directed mutagenesis](#) on the heparan [sulfate](#) receptor footprint (22). This chimeric vector encompassing the serotypes [AAV2](#) and 8 (AAV2i8) transduced a wide range of muscle groups in the murine forearms and legs as well as cardiac, intercostal and facial muscles (22). These encouraging results paved the way for an upcoming clinical gene therapy trial with AAV2i8 in 12 patients with advanced HF (7). AAV2i8 ability to efficiently transduce not only cardiac muscle, but also various skeletal muscle groups with 2–3 times higher transduction efficiency relative to the liver was definitely a major advancement in the field, particularly promising for the treatment of some forms of muscular dystrophy, as better detailed in the next paragraph.

Genetic correction of Duchenne muscular dystrophy/Duchenne cardiomyopathy

Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy and is caused by gene mutations leading to deficiency of the protein dystrophin, a large structural protein that anchors the inner surface of muscle sarcolemma to F-actin (23). The structure of sarcolemma lacking dystrophin is unstable, causing muscle damage and triggering an

inflammatory response, which leads to further damage, necrosis, and fibrosis. Dystrophin is mainly expressed in cardiac and skeletal muscle, which are the first organs to be affected by degeneration and weakening, yet DMD is a multi-system disorder resulting in severe physical disability and premature death (24–25).

The current therapy of DMD is merely palliative, based on corticosteroids and physical therapy (26). Given the monogenic origin of this disease, gene therapy is the obvious, ideal approach for a radical cure (27). However, despite the remarkable number of promising studies performed in animal models, the first clinical trials are still in the phase of planning and recruitment (28–29). As for other types of HF, AAVs are considered the vectors of choice also for DMD (30–32), but their key limitations emerge even more evident in this case, due to the necessity of whole body transduction and life-long transgene expression (29). To date, only one clinical trial has been completed, examining intramuscular injection of a hybrid AAV2.5 vector in six patients. This therapy proved safe, however the patients did not express significant levels of dystrophin (33,34). Despite the predictable difficulties, the extensive data accumulated in preclinical studies in non-human primates (35,36) and dogs with naturally occurring DMD (37–39) will likely prompt new clinical trials (34, 40–42).

A major hurdle for gene therapy of DMD is the large size of the dystrophin gene, which cannot be packaged as full-length coding sequence in AAVs (43). Clever solutions have been tested to overcome this obstacle. One of them is the trans-splicing of dual or triple AAV vectors, which consists of dividing a large gene and then packaging in and delivering it by two or three AAVs. The complete gene is then expressed by dual or triple trans-splicing (44–46). Although this approach has been established, the transduction efficiency is still too low to achieve therapeutic levels of functional proteins. More recently, the dual AAV therapy to deliver a miniaturized form of the dystrophin gene (mini-dystrophin) has been successfully tested in a canine model, thus providing the first clear evidence of clinical translation potential (38).

Another approach proposed to treat DMD is genome editing for repair of the defective gene at its locus. The cured gene would then retain its native regulation. To date, nucleases and clustered regularly interspaced short palindromic repeats (CRISPRs) systems have been tested in several models of disease. Nuclease-mediated gene editing by using zinc-finger nucleases, TALENs (transcription activator-like effector nucleases) and meganucleases have proved efficacious for the treatment of muscle disorders in experimental and even clinical studies (47–51). Very recently, the CRISPR/Cas9 system has been delivered via AAVs to mice with dystrophin deficiency caused by a spontaneous mutation of the dystrophin gene (43). The aim was to remove exon 23 from the dystrophin gene, leading to the expression of a partially functional dystrophin in skeletal myofibers and cardiac muscle and enhance muscle force (52–54).

Exon skipping is an alternative method of gene editing based on RNA splicing system, which can be briefly described as follows. Under normal conditions, the native RNA transcribed from a gene is processed by RNA splicing that involves the removal of the introns. The mature mRNA consists of the remaining sequences named exons. By removing an **exon** that lies near the mutation, the downstream reading frame can be corrected and

partially functional dystrophin can be restored. Exon skipping can be manipulated by delivering antisense oligonucleotides (AONs) that target particular sequences in exons. This has been tested with satisfactory results in small and large animal DMD models, followed by the failure of a number of clinical trials (29). However, after phase II/III clinical trials resulting in 23% increase in dystrophin positive fibers, Eteplirsen (AON inducing exon 51 skipping) (55) was approved to by FDA in 2016. Sadly, Eteplirsen targets only 13% of DMD cases. Other clinical trials based on exon skipping are challenged by the higher inefficiency of this system (56). Interestingly, it was shown that Dantrolene, a drug currently used for the treatment of the malignant hyperthermia, can enhance antisense-directed exon skipping (57). This might be a promising strategy to improve AON treatment and reduce the dose of delivered oligonucleotides, therefore lowering costs and potential toxicity.

Despite promising results in animal models, clinical trials are not showing solid and consistent results. The patient phenotype is much more complex than experimental models. For instance, the sarcolemma integrity in muscle fibers, essential for efficient and long-term gene therapy, is severely and variously compromised in DMD patients (58,59). Procedural risks and observed toxicity (60) as well as potential side effects, such as myositis and contractures (61) following the gene delivery should be considered. Precise clinical characterization, individualization and combined therapies could prove key to overcome existing limitations (51).

Other strategies with promising translational potential

AC6 (Adenylyl cyclase) catalyzes the conversion of ATP to cyclic adenosine monophosphate (cAMP), a molecule that is essential for cardiac function. cAMP is generated in response to β -adrenoceptors stimulation by catecholamines and modulates excitation-contraction coupling leading to positive inotropic effect (62). In a swine model of HF, AC6 gene transfer by adenoviral vectors improved heart function and reversed pathological LV remodeling (63). Cardiac AC6 overexpression prolonged life in mice with genetic cardiomyopathy (64), didn't cause unbridled cAMP generation and reduced arrhythmias (65). Recently, the safety and efficacy of intracoronary delivery of AC6 carried by adenoviral vectors has been assessed in patients with symptomatic HF (EF 40%) in a multicenter, double-blind, placebo-controlled, phase 2 study (66). This intervention improved LV function more than the standard HF therapy. Larger trials are expected.

S100A1 is a protein that modulates sarcoplasmic reticulum Ca^{2+} cycling and mitochondrial function through interactions with the ryanodine receptor, SERCA2 and mitochondrial F_1 -ATPase activity. It exerts anti-hypertrophic, inotropic and antiarrhythmic effects and attenuates energy depletion in HF (67,68). S100A1 is significantly downregulated in human as well as various animal models of HF (67–69), therefore this alteration is a potential target for corrective gene therapy. In fact, AAV9-S100A1 retrograde coronary gene transfer in myocardium rescued cardiac contractile function in a preclinical large animal model (69). Moreover, AAV6-S100A1 is safe (70). In 2016 the Dutch company UniQure has developed gene therapy with AMT-126 (AAV-S100A1) to selectively restore cardiac deficiency of S100A1 (71). MicroRNA-138 can be considered as another related target, since it causes hypoxia-induced endothelial cell dysfunction by downregulating S100A1 (72). Transfection

of endothelial cells with a miR-138 mimics reduced S100A1–3'UTR reporter gene expression, while transfection with an anti-miR-138 prevented the hypoxia-induced downregulation of the reporter gene (72). Experiments in vivo will be necessary to test the real efficacy of this strategy.

VEGF-B (Vascular Endothelial Growth Factor-B) selectively binds VEGFR-1, one of the five receptors of the VEGF family (73). VEGFR-1 is not involved in angiogenesis, but mediates the strong cytoprotective/antiapoptotic effects of VEGF-B (73,74). Such characteristics render VEGF-B an ideal candidate for gene therapy of dilated cardiomyopathy, a form of HF characterized by a remarkable increase in cardiac cell apoptosis (75,76), which is directly proportional to the rapidity of clinical deterioration (77). Despite its lower prevalence compared to ischemic cardiomyopathy as a cause of HF, dilated cardiomyopathy is particularly malignant and refractory to pharmacological treatments, accounting for approximately 50% cases of heart transplantation in the US (78). The ideal cure of it would consist of halting cardiac apoptosis, while, different from ischemic HF, angiogenesis is not necessary or even detrimental. After the first encouraging results in a rat model of myocardial infarction (79), we have tested cardiac gene delivery of VEGF-B carried by AAV9 vectors in a preclinical dog model of tachypacing-induced dilated cardiomyopathy. Both of direct intramyocardial injection (80) and intracoronary infusion (81) of AAV-VEGF-B proved markedly beneficial, causing a delay in the onset of cardiac decompensation and opposing the development of numerous functional, histological and molecular alterations, including apoptosis. Such positive results in a well-established experimental large animal model prompted us to start a pilot veterinary clinical trial in dogs with spontaneous dilated cardiomyopathy (personal communication). The results of this ongoing study will provide indications about the real potential of VEGF-B gene therapy.

SDF-1 (stromal cell-derived factor-1) was shown to be critical in cardiac stem cell therapy, as it affects stem cell homing, cardiomyocyte survival and ventricular remodeling in animal models of acute myocardial infarction or chronic HF (82). Later evidence suggested that SDF-1 alone is sufficient to induce cardiac repair (83). A first non-randomized, open-label clinical trial tested SDF-1 direct trans-endocardial delivery with the plasmid vector in seventeen subjects with ischemic cardiomyopathy. (84) Besides the modality of delivery, i.e. intramyocardial instead of intracoronary infusion and naked DNA instead of AAV-carried transgene, this strategy differed from those adopted in the SERCA2a and AC6 trials relatively to the mechanism of action: SDF-1 is a cytokine with no direct effects on cardiac function, but operates by stimulating endogenous repair processes including angiogenesis. The initial promising results prompted a Phase 2 blinded, placebo-controlled, multicenter trial that included 93 subjects with stable symptomatic HF (ejection fraction <40%) of ischemic etiology (85). This study failed to achieve its primary endpoint of an improved composite score at 4 months after treatment. However, patients with the lowest ejection fraction, who received the highest dose of pSDF-1, showed a 7-unit increase in ejection fraction compared with a 4-unit decrease found in placebo patients at 12 months. These results led the authors to the conclusion that the treatment is more specifically effective in patients with very depressed ejection fraction, which might represent the target population in

subsequent trials. The following paragraph will review more extensively other new strategies of gene therapy for cardiac regeneration.

A new frontier: gene therapy to stimulate cardiac regeneration

Cardiac regeneration has been a leading topic in cardiological research over the past 15 years. The long chased dream is to block the evolution of post-ischemic pathological remodeling and progressive dysfunction, i.e. the most frequent form of HF, by replacing areas of dead myocardium with fully functional and vascularized newly formed tissue. The main strategies proposed to date are based on transplantation of pluripotent stem cells, such as myoblasts deriving from skeletal muscle or cardiac and non-cardiac progenitor stem cells differentiating into cardiomyocytes or other mature cell lines (86–89). Extensive research in this field has already led to clinical applications; nonetheless, the effective role and retention of transplanted cells in injured hearts remain controversial (89,90).

An alternative approach would consist of stimulating the proliferation of the surviving/viable tissue in injured hearts. This idea seems much more arduous, since it is based on the assumption that mature cardiomyocytes, which have very low renewal rate soon after birth (91), and/or other resident cell types might proliferate and reconstruct functional myocardial tissue formed by muscular parenchyma, vessels, stroma and organized conduction system. However, over the past few years, parallel studies conducted in different laboratories on the overexpression of various cell cycle regulators indicated that the stimulation of mature endogenous cardiomyocyte proliferation is a realistic option (5) and targeting the intracellular signals involved in these processes might prove a novel strategy of gene therapy for HF. For example, transgenic overexpression of cyclin A2, which is normally silenced in postnatal hearts, was reported to stimulate cardiomyocyte proliferation (92) and to be beneficial after myocardial infarction (93). Forced overexpression of the cyclin-dependent kinase 2 also caused an increase in nuclear antigens associated with DNA replication in adult mouse transgenic hearts, followed by a transient increase in cardiomyocyte proliferation and the appearance of less differentiated mononuclear cardiomyocytes (94). Transgenic mice overexpressing the early G1 cyclins displayed sustained DNA synthesis, but abnormal patterns of multinucleation (95), whereas the overexpression of cyclin D2 induced regenerative growth and infarct regression (95,96). Taken together, these studies in transgenics indicate the potential to achieve postnatal cardiomyocyte DNA synthesis and nuclear division. However, they did not provide conclusive evidence that karyokinesis was followed by cytokinesis and, most importantly, by proliferation of these cells. On the other hand, it was reported that the deletion of the homeodomain transcription factor Meis1, a regulator of normal cardiac development, was sufficient to extend the postnatal proliferative window of cardiomyocytes and to re-activate cardiomyocyte mitosis in the adult heart, with no adverse effects on cardiac function (97). Moreover, it was recently shown that Tbx20, a T-box gene required for cardiomyocyte proliferation during heart development, can act as a transcriptional repressor of Meis1 (98). Tbx20 overexpression increased cardiomyocyte proliferation and preserved cardiac function maintenance after myocardial infarction in adult mice, therefore supporting Meis1 potential as a therapeutic target for cardiac regeneration (98).

An alternative research direction has focused on the role of miRNAs in cardiac cell proliferation. It was found in mice that the administration of antagonists of the microRNA-15 family was associated with an increased number of mitotic cardiomyocytes in the post-natal life (98). This study hinted at the possibility that micro-RNAs, important modulators of mRNAs translation, can be targeted to obtain cardiac regeneration. A functional screening of the whole human genome miRNA library led to the identification of specific miRNAs capable of promoting cardiomyocyte proliferation (99). The most effective ones, miRNA-590 and miRNA-199, were then delivered via AAV to mouse hearts in vivo, where they induced cardiac regeneration and consequent improvement of cardiac function after myocardial infarction (99). Therefore, this study showed for the first time that post-infarct cardiac regeneration could be obtained, in vivo, by delivering molecules (not cells) with a classical method of gene therapy. Subsequent investigations identified the Hippo pathway as a target of pro-regenerative miRNAs (Figure 1) (100). The Hippo pathway is a kinase cascade controlling organ size in mammalian organisms through the regulation of cell size, proliferation, apoptosis, survival, differentiation and migration in developing organs (101). This signaling pathway, which prevents adult cardiomyocyte proliferation and regeneration postnatally, is upregulated in human HF. (102) The main kinase of the Hippo pathway and the only one known to promote tissue growth is the transcriptional co-activator YAP (Yes-Activated Protein). Following phosphorylation of the Hippo pathway components *Mst*, *Lats* and *Salv*, the final step of activation is the phosphorylation and cytoplasmic sequestration of the transcriptional cofactor Yap. This leads to the binding of DNA by Tead factors (102). Recently, the Hippo pathway was “turned off” by deleting *Salv* in a mouse model of ischemic HF (103). Hippo-deficient cardiomyocytes displayed increased expression of pro-proliferative and stress response genes, such as the mitochondrial quality control gene Park2. Treated rodents presented reduced fibrosis and better recovery of heart function. *Salv* *Lats1* and *Lats2* deletion can also improve heart function after myocardial infarction in small animal models (104–106).

The capacity of various miRNAs to stimulate cardiomyocyte proliferation was further supported by later studies. It was shown that overexpression of the miRNA-17–92 cluster in transgenic mice induced cardiomyocyte proliferation either during embryonic or postnatal stages, resulting in high heart/body weight ratio and increased thickness of the left ventricular wall. Moreover, infarcted hearts of these transgenic mice displayed smaller scar size and improvement of cardiac function (107). Others reported that the treatment of neonatal cardiomyocytes with miR-29a, miR-30a or miR-141 inhibitors induces a higher percentage of cycling cells (108). The cluster miR-302–367 was identified as a driver of cardiomyocyte reentry in the cell cycle and inducer of cardiac regeneration after myocardial infarction, at least in part due to inhibition of the Hippo pathway (100). The miR-302–367 cluster was shown to repress the kinases *Mst1*, *Lats2* and *Mob1b*: the loss of these essential components in the developing mouse heart caused increased proliferation in cardiomyocytes.

The role of miRNAs in the regulation of cardiomyocyte proliferation and the potential exploitation of the related mechanisms for future clinical applications is currently a hot topic (109–114) and a new exciting perspective for gene therapy of HF. Systematic tests in clinically relevant animal models are warranted. Our group has obtained very encouraging preliminary evidence in infarcted pigs (115).

CONCLUSIONS

Gene therapy for HF is rooted in pioneer studies published almost 30 years ago and has been characterized by phases of hype and disappointment. The failure of the first clinical trial launched in 2012 should not induce investigators to abandon this area of research. In fact, in 2016 4D Molecular Therapeutics made available the 3rd generation AAV vectors for gene delivery to treat HF (116). New molecular targets with remarkable curative potential have been and are being identified. The delivery of non-coding nucleic acids such as microRNAs is proving effective to induce cardiac regeneration, thus displaying a curative property so far attributed only to stem cells. HF is a complex, multi-factorial syndrome and, with the exception of the monogenic heritable forms, the potential therapeutic targets are numerous, hence it is difficult to identify the “magic” one. This explains in part the difficulties encountered by gene therapy for HF. It should be pointed out that also traditional therapeutic approaches are plagued by similar problems. Investigators in the area of gene therapy should persist in their efforts, especially because this therapeutic strategy can still reveal an unexpressed potential.

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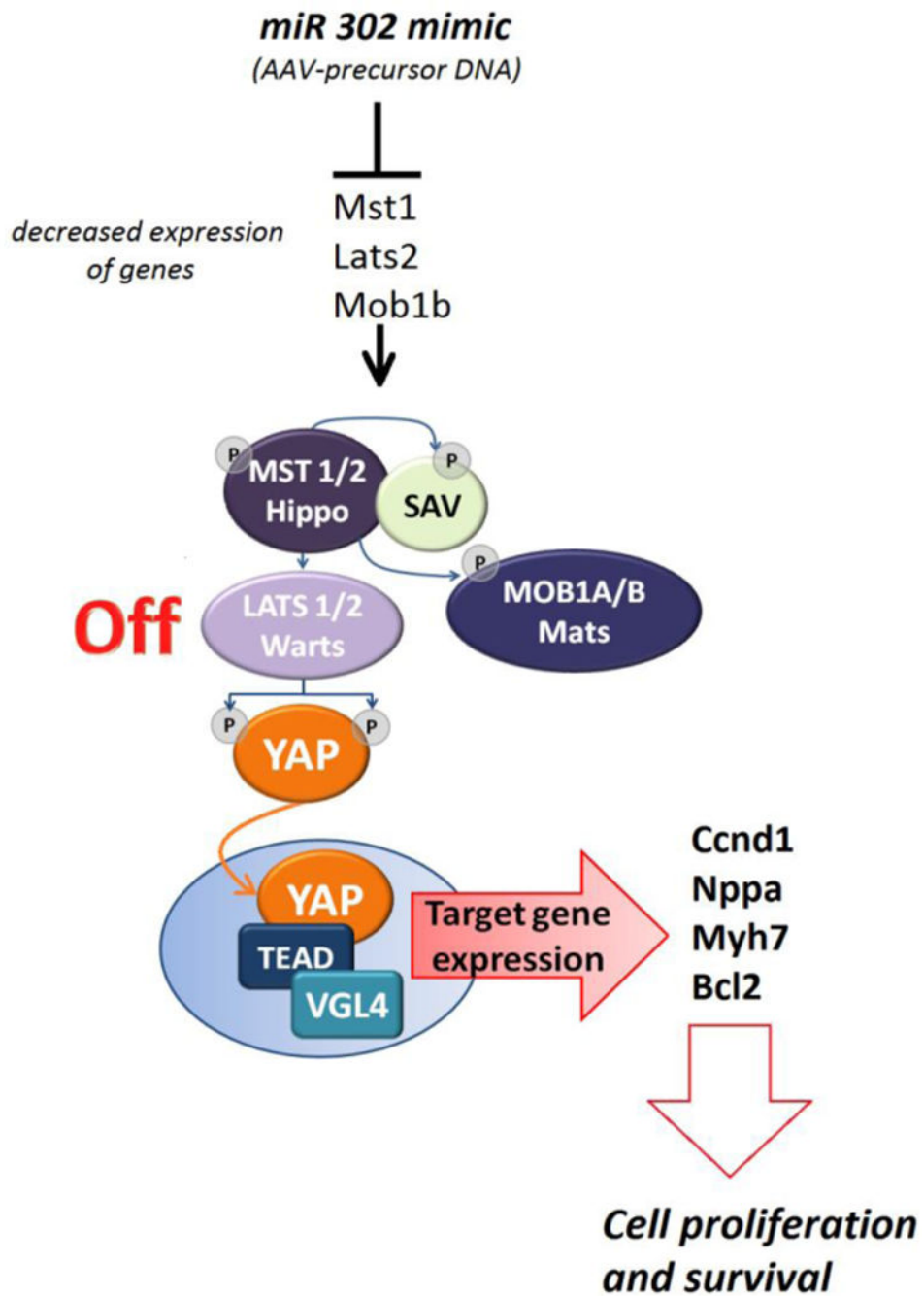


Figure 1: Several miRNAs may target the key kinases of hippo cascade: Mst, Lats, Mob. The loss of these essential components leads to transcriptional co-activator YAP (Yes-Activated Protein) translocation and accumulation in the nucleus, where it displaces VGL4 (the transcription cofactor vestigial-like protein 4), forms a complex with TEADs (TEA domain-containing sequence-specific transcription factors) and activates the transcription of its target proliferative genes: Ccnd1 (cell proliferation), Nppa, Myh7 (fetal gene program), Bcl2 (anti-apoptosis).

Hippo pathway kinase phosphorylation cascade: Hippo (Mammalian sterile-20-like kinases type 1 and type 2 or MST1, MST2), Salvador (SAV), Warts (Large tumor suppressor-LATS 1/2) and Mob as tumor suppressor (Mps-one binder kinase activator-1; MOB1).

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