Rapamycin inhibits human in stent restenosis vascular smooth muscle cells independently of pRB phosphorylation and p53

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Abstract

**Objective:** Drug-eluting stents containing the immunosuppressant rapamycin markedly inhibit in stent restenosis (ISR). However, the molecular mechanisms that underlie its effect on ISR-derived vascular smooth muscle cells (VSMCs), as opposed to normal VSMCs, are unknown. Specifically, as ISR-VSMCs have altered cell cycle regulation, rapamycin may arrest these cells via novel molecular pathways.

**Methods:** We isolated human VSMCs from sites of ISR, and examined the effect of rapamycin on cell proliferation using MTT assay, time lapse videomicroscopy and flow cytometry. Regulation of G1–S transition was examined using Western blotting, and cell size and protein synthesis examined using flow cytometry and collagen assay, respectively. The requirement for pRB and p53 was examined using ISR VSMCs expressing E1A and a dominant negative p53, respectively.

**Results:** ISR-VSMC proliferation was potently inhibited by rapamycin. Arrest was confined to G1, as cell proliferation (but not cell size) of S/G2-arrested cells was unaffected by rapamycin. Moreover, ISR-VSMC lines generated with disrupted p53 or pRB function still arrested in the presence of rapamycin, suggesting that these genes are dispensable for rapamycin-induced arrest. Significantly, rapamycin completely inhibited the phosphorylation of p70S6K, an mTOR-regulated kinase implicated in the control of proliferation, but had no effect on collagen or total protein synthesis.

**Conclusions:** We demonstrate that rapamycin is a potent inhibitor of ISR VSMC proliferation during G1. Rapamycin’s action does not require p53 or pRB. We show that p70S6K is markedly inhibited in rapamycin-arrested ISR cells, suggesting that regulation of its upstream kinase, mTOR, is important for the control of proliferation in ISR cells.

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**Keywords:** Restenosis; Stents; Smooth muscle; Atherosclerosis

1. Introduction

Despite the increasing use of stents for coronary intervention in stent restenosis (ISR) remains a significant clinical problem. ISR is caused by the accumulation of vascular smooth muscle cells (VSMCs) and their associated extracellular matrix, resulting in neointima formation. Treatment by stenting, balloon angioplasty or a variety of other techniques all result in high rates of re-intervention. However, drug-eluting stents containing the immunosuppressive rapamycin (Sirolimus) have shown encouraging reductions in ISR in de novo lesions [1,2].

Rapamycin has been shown to inhibit the proliferation and promote the differentiation of normal VSMCs in vitro, and also to inhibit the proliferation of VSMCs in ISR animal models. What remains contentious is the molecular mechanism through which rapamycin achieves its clinically useful effect. Activation of VSMCs via growth factors or arterial injury is associated with decreased expression of the cyclin dependent kinase inhibitor (cdki) p27 [3]. p27 inhibits the activity of the G1–S kinases cdk2
and cdk4. Activation of both cdk4/6 and cdk2 is required for hyperphosphorylation of pRB and subsequent release of E2F transcription factors for progression into S-phase. Some studies suggest that rapamycin blocks pRB function via activation of cdkis [4]. Indeed, transcriptome analysis indicates that rapamycin downregulates many genes associated with G1–S transit including cyclin D isoforms, E2F-1 and E2F target genes [5]. Others claim there is no change in pRB phosphorylation, and therefore rapamycin cannot work via this protein [6]. The specific role of p27 in rapamycin-induced arrest in VSMCs is also unclear [7–9]. Rapamycin treatment of VSMCs induces p27 expression, and rapamycin resistance has been shown to correlate with reduced p27 expression [7]. Rapamycin also inhibits cdk/cyclin activity in proliferating VSMCs after vessel injury [10], an effect that should result in hypophosphorylation of pRB and growth arrest. However, p27 knockout animals show rapamycin-mediated inhibition of neointima formation, suggesting that p27 function is not required [8].

Rapamycin, attached to an intracellular protein FKBP12 [11], binds and inhibits a PI3 kinase (PI3K)-related protein, mammalian Target Of Rapamycin (mTOR). mTOR causes both the phosphorylation and activation of 
\[ p70^{S6R} \], a kinase implicated in cell proliferation [12], and the phosphorylation and inactivation of 4E-BP1, a protein that inhibits the translation of 5’ cap mRNAs [13–15]. Growth factor activation of mTOR in VSMCs promotes cell cycle-related protein synthesis, in part through inactivation of 4E-BP1, while rapamycin promotes the activation of 4E-BP1 [13–15] and inhibits 5’cap mRNA translation. Interestingly, 4E-BP1 null cells still arrest in the presence of rapamycin [16,17]. Thus, the effect of translation inhibition via 4E-BP1 remains unclear. Importantly, rapamycin may promote the differentiation of VSMCs into a more contractile, less proliferative phenotype. This requires new protein synthesis and functional p70S6K, and occurs despite active 4E-BP1 [18]. In line with these observations, a number of papers suggest p70S6K activity is central to the regulation of proliferation [12,19]. However, whether this is a downstream effect of mTOR or the PI3K-Akt/PKB pathway, or both, has yet to be resolved [12,19].

We have previously shown that ISR-VSMCs have profound differences in expression profiles of proteins involved in G1 to S-phase progression [20]. In particular, ISR-VSMCs have increased expression of cyclins E and A, p21 and p16, but normal p27 levels compared to normal coronary VSMCs [20]. Because rapamycin has been studied only in normal VSMCs, it is essential to determine rapamycin’s effect on ISR VSMCs, its actual target in diseased arteries. To investigate growth arrest via rapamycin in ISR cells, we also examined the effect of rapamycin on ISR VSMCs engineered to express defective p53 (ISR-DN-p53) [21] or non-functional pRB (ISR-E1A-ER™).

2. Methods

2.1. Tissue collection and cell isolation

2.1.1. Human tissue collection and cell isolation

Human tissue collection was approved by the Local Ethical Committee, and consent obtained from patients before tissue harvest. The investigation conforms with the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997; 35:2–4). Normal aortic VSMCs were explanted from patients undergoing cardiac transplantation for non-ischaemic cardiomyopathy (n=4). ISR specimens were obtained by directional coronary athrectomy from sites of angiographically confirmed ISR from patients with stable angina (n=4). All VSMCs were isolated by enzyme digestion and explant outgrowth [22,23] and cells cultured in M199 medium+20% foetal calf serum (FCS), 100 IU/mL penicillin, 100 mg/L streptomycin, 250 mg/L amphotericin B and 4 mmol/L L-glutamine. Individual patient VSMC cultures were not pooled. Characterisation of cells as VSMCs was as previously described [24].

2.1.2. Retrovirus infection of human ISR-VSMCs

Human ISR-VSMCs stably expressing dominant negative p53 (DN-p53), a regulated adenovirus E1A 12S protein (pBabepuro E1A-ER™, kind gift of Dr A Fanidi, Ca) or vector alone were generated using amphotropic retroviruses as previously described [21,25]. pBabepuro E1A-ER™ was transfected into the ecotropic packaging cell line BOSC23, and the 48-h supernatant used to infect an amphotropic virus-generating cell line (EnvAmp12). ISR-VSMCs were infected with supernatant from these cells at 48 h, and selected in 10 μg/ml puromycin (Sigma). E1A-ER™ expression in pooled cells was confirmed by Western blot. E1A activity was induced by 30 min preincubation with 70 nM 4-hydroxytamoxifen (4-OHT) (Sigma).

2.1.3. MTT Assay

Cells were cultured on 96 well plates in medium containing 20% FCS with increasing concentrations of rapamycin (Sigma) for 16 or 48 h and cell number assessed using MTT assay with absorbance at 570 nm measured by spectrophotometry.

2.1.4. Time-lapse videomicroscopy

Rates of proliferation and apoptosis were determined as described [26] for 50% confluent, non-overlapping cells using time-lapse videomicroscopy. Cell proliferation was scored at septation of daughter cells and cumulative % mitosis in each culture calculated for each 6 h time period.

2.1.5. Flow cytometry

Cell cycle profiles were determined by propidium iodide staining of DNA content and flow cytometry. Cells were
synchronised to G₀ by incubation in 0% FCS/DMEM for 48 h and to S-phase by double thymidine block in 2 mM thymidine (Sigma) as described [26]. Cell size was determined by assessing forward scatter at each cell cycle stage as defined by PI intensity.

2.1.6. Western blotting and immunoprecipitation

Protein isolation, electrophoresis and blotting were performed as described [27]. All experiments were repeated on at least 3 separate occasions. Primary antibodies used were mouse anti-cyclin A(554174)-cyclin D(554203), -cyclin E(554182), -p21(554262), -p27(554069), -E2F(554213), -p16(554070) and -E1A (554155)(all 1/1000 (v/v)), mouse anti-pRB(554136) (1/500 (v/v))(all from Pharmingen), Rabbit anti-p70S6k(Sc-230), -PCNA(Sc-7907) and -Cdk4(Sc-601), and goat anti-4E-BP1(Sc-6025) (all 1/500 (v/v)) were purchased from Santa Cruz Biotechnology. Appropriate secondary horseradish peroxidase-linked antibodies (Amer sham) or (Santa Cruz Biotechnology) were used at between 1/1000 (v/v) and 1/10000 (v/v). For cyclin D or Cdk4 immunoprecipitation, Protein G beads (Sigma) were incubated for 2 h with 2 μg antibody (Santa Cruz) in lysis buffer and washed 3 × before addition of total protein lysate.

2.1.7. In vitro kinase assay

Cdk4 immunoprecipitation of total cellular extracts were used to phosphorylate a pRB-C fusion protein (Cell Signaling Technologies) as per manufacturer’s protocol. Cdk4/Cyclin D₁ complex (kind gift of M.D. Garrett, CRC, Cambridge) was used as a positive control.

2.1.8. Measurement of protein synthesis

For measurement of new collagen and global protein synthesis, cells were cultured in 1% FCS±rapamycin for 5 h, washed and pulsed with 14 μCi ³H-proline (Amersham) in serum-free media supplemented with 150 nM ascorbic acid, 0.04% proline, 0.1% BSA (Sigma)±rapamycin as before. Cells were counted and sonicated. Total protein was precipitated in 10% TCA/0.1% BSA/0.04% proline and then digested for 90 min with Type III Collagenase (50 U; Sigma). Protein was re-precipitated in 20% TCA/tannic acid, centrifuged to separate collagenase-digestible (supernatant) from non-digestible (pellet) protein. Supernatants and pellets were counted separately in a scintillation counter. Percent collagen was calculated using a formula correcting for non-collagen proline content (% collagen=[ratio/5.4×(1−ratio)]+ratio] where ratio=cpm sup+cpm sup+pellet). De novo protein synthesis was expressed as total counts from supernatant and pellet per 10⁶ cells.

2.2. Statistical analysis

Results are expressed as mean±S.E.M. Analysis of significance was by ANOVA.

3. Results

3.1. Rapamycin induces growth arrest in ISR-VSMCs

To examine how rapamycin inhibits proliferation in ISR, we isolated ISR VSMCs from directional coronary athere cto my specimens (n=4) [20]. The characterisation of basal proliferation and phenotype, together with their expression of cell cycle regulator proteins has been published previously [20,21]. We also generated stable VSMC pools expressing either dominant negative p53 [21] (ISR-DN-p53) or cells with defective pRB function (ISR-E1A- ER™). This construct expresses a fusion protein of E1A with a mutated version of the ligand-binding domain of the human oestrogen receptor (ER), that can be activated by 4-hydroxytamoxifen (4-OHT). The E1A protein disrupts pRB function, inducing E2F activity [28] and abrogates p27 function [29,30]. Ablation of pRB function in human VSMCs induces apoptosis; thus stable cell pools with a 4-OHT-regulatable adenoviral E1A protein enables pRB function to be disrupted in a controlled manner. ISR-E1A-ER™ cells treated with 4-OHT survive for 18–24 h before pRB loss induces apoptosis. ISR VSMCs expressing DN-p53 showed low endogenous p53 activity (Fig. 1A); we have previously shown that these cells show defective p53-induced growth arrest and apoptosis [21]. ISR-E1A-ER™ cells expressed E1A (Fig. 1B), and 4-OHT activation of these cells increased E2F-1 and its target genes Cyclin E and PCNA (Fig. 1C). These studies confirm that these vectors are both expressed and active in ISR cells.
ISR-VSMCs treated with increasing doses of rapamycin (Fig. 2A) demonstrated a dose-dependent reduction in MTT activity, which plateaued, indicating no toxicity over this dose range. Inhibitory effects were maximal at 50 ng/ml. Both ISR-DN-p53 and ISR-E1A-ER™ VSMCs were susceptible to rapamycin, the latter to a lesser extent. Treatment with 4-OHT in cells not expressing E1A-ER™ was without effect (not shown). The sensitivity of all cell lines indicates that neither p53 nor pRB are required for rapamycin’s effect. To confirm this, and to exclude the

![Graphs showing MTT activity and mitoses](https://example.com/fig2graphs.png)

**Fig. 2.** Rapamycin is a potent inhibitor of cell proliferation in ISR-VSMCs. (A) ISR, ISR-DN-p53 or ISR-E1A-ER™ VSMCs in exponential phase culture were treated with increasing concentrations of rapamycin and relative cell number assessed by MTT assay after 48 (ISR, ISR-DN-p53) or 16 h (E1A-ER™). Data are means±S.E.M., *p<0.05 (n=3). (B) Time-lapse videomicroscopic analysis of cumulative % mitoses in ISR, ISR-DN-p53 or ISR-E1A-ER™ VSMCs over time in the presence or absence of 50 ng/ml rapamycin. Data are means±S.E.M. (n=3).
Fig. 3. Rapamycin effects are restricted to G1. Flow cytometric cell cycle analysis of ISR or ISR-DN-p53 VSMCS after (A) restimulation from serum starvation-induced growth arrest or (B) after double thymidine block and release, in the presence or absence of rapamycin (n=3). Individual cell cycle phases and % in each phase are marked.
possibility that rapamycin affected the ability of the cell to metabolise MTT, cells were treated with 50 ng/mL rapamycin and analysed by time-lapse videomicroscopy. Rapamycin significantly slowed cell proliferation in ISR and ISR-DN-p53 VSMCs (Fig. 2B), with no induction of apoptosis (not shown). ISR-E1A-ER™ VSMCs underwent apoptosis beyond 18 h when E1A was active. However, rapamycin significantly reduced mitoses at earlier time points (12 and 18 h, Fig. 2B, \( p=0.005 \) and 0.035, respectively).

3.2. The growth suppressive effect of rapamycin occurs in \( G_1 \) in ISR-VSMCs

To examine the effect of rapamycin on cell cycle entry, cells were arrested in serum-free conditions for 48 h, re-stimulated with 20% FCS+rapamycin and DNA content analysed by flow cytometry at 18 h. Although DN-p53 cells showed relatively higher S+G2/M phase percentages in 20% FCS or low serum conditions, consistent with a growth suppressive effect of p53, rapamycin-treated ISR or ISR-DN-p53 VSMCs cells were slower to enter S-phase, confirming a \( G_1 \) point of action (Fig. 3A). Since ISR cells have a distorted cell cycle, that is critically dependent upon cdk2/cyclin E [20], it is plausible that rapamycin may also arrest these cells beyond \( G_1 \). Therefore, cells were synchronised at \( G_1/S \) using a double thymidine block, released and treated+rapamycin for 18 h. Treatment of cells with thymidine causes an early S-phase block that can be released upon drug removal. A sequential (double) block is used to ensure as many cells as possible arrest in S-phase. Fig. 3B shows that cell cycle transition after thymidine removal was not affected by rapamycin. Thus, rapamycin will inhibit passage through \( G_1 \), but has no detectable effect on other phases of the cell cycle. ISR-E1A-ER™ VSMCs treated with 4-OHT underwent apoptosis with thymidine, and therefore could not be studied using this system.

3.3. Rapamycin increases p27 and p27/cyclin D complexes

To examine further the molecular action of rapamycin, ISR and ISR-DN-p53 VSMCs were treated with rapamycin and protein lysates analysed at 48 h by Western blotting (Fig. 4). Rapamycin increased p27 levels by 90% in ISR cells or 40% in ISR-DN-p53 cells, but no changes were seen in expression of cdk4, cyclin D, Cyclin E, p53, p16 or p21 were evident. Immunoprecipitation and in vitro kinase assay demonstrated that rapamycin increased p27 association with cyclin D by 60% in ISR and ISR-DN-p53 cells, and reduced cdk4-associated kinase activity. However, no changes in

![Fig. 4. Rapamycin increases p27 and p27/cyclin D complex formation. ISR or ISR-DN-p53-VSMCs were treated with 50 ng/ml rapamycin and protein lysates isolated at 48 h for Western blotting, immunoprecipitation of Cyclin D followed by blotting with p27 (Densitometry ISR-VSMCs+rapamycin \( p=0.01 \); ISR-DN-p53-VSMCs+rapamycin \( p=0.037; n=3 \)) or in vitro kinase activity (Densitometry ISR-VSMCs+rapamycin \( p=0.005 \); ISR-DN-p53-VSMCs+rapamycin \( p=0.047; n=3 \)). Note the absence of any change in pRB phosphorylation status. Western blots of normal aortic and ISR-E1A VSMCs after rapamycin treatment are shown for comparison.](image-url)
pRB phosphorylation states were seen. We also examined changes in protein expression in normal aortic VSMCs and ISR-E1A VSMCs. Compared with ISR cells, normal aortic VSMCs showed a greater rise in p27 and Cyclin D-associated p27 than ISR cells (230% and 160%, respectively). A marked suppression of cdk4-associated kinase activity was also seen, although again, pRB phosphorylation was not altered. Despite a marked suppression of proliferation, rapamycin did not affect G1–S regulator protein or cdk4-associated kinase activity expression in ISR-E1A cells. The lack of effect of rapamycin on pRB phosphorylation in any cell type, and the anti-proliferative effect that it is independent of pRB.

3.4. The effect of rapamycin on cell size

mTOR is a regulator of cell size, an effect mediated in part by p70S6K and 4E-BP1[31]. ISR-VSMC size increased during cell cycle transit in both treated and untreated cultures examined by flow cytometry. However, rapamycin-treated cells were smaller overall than untreated controls (Fig. 5A), which reached significance at G2/M ($p<0.05$ ($n=3$)).

The effects of rapamycin on cell size and also the anti-proliferative effects of this drug in the absence of changes in pRB phosphorylation suggest that alterations in rapamycin target proteins may mediate these effects independent of G1–S regulator expression. In particular, recent studies suggest that 4E-BP1 and p70S6K activity may be central to the regulation of proliferation [12,19]. Western blotting (Fig. 5B) showed that in ISR-VSMCs stimulated from quiescence, 4E-BP1 phosphorylation reverted to quiescent levels when treated with rapamycin, and p70S6K phosphorylation was markedly inhibited by rapamycin. This profound effect of rapamycin on 4E-BP1 and p70S6K phosphorylation was also seen in ISR-E1A cells, further confirming an anti-proliferative effect that it is independent of pRB.

3.5. The effect of rapamycin on protein synthesis in ISR-VSMCs

The ISR neointima is composed predominantly of extracellular matrix; thus inhibition of matrix synthesis may exert a profound effect on neointima formation. We examined the effect of rapamycin on incorporation of radiolabelled hydroxyproline in ISR-VSMCs as a surrogate marker for collagen synthesis and total protein content of cells. Surprisingly, despite hypophosphorylation of 4E-BP1 and p70S6K, rapamycin did not inhibit new collagen or
protein synthesis (Fig. 6). Raising doses of rapamycin to 500 ng/ml was also ineffective; in comparison, incubation with 10 µg/ml cycloheximide completely inhibited new protein and collagen synthesis (not shown). To confirm whether this result was exclusive to ISR cells, we also tested rapamycin on normal human aortic VSMCs—no effect of rapamycin on protein or collagen synthesis was seen.

4. Discussion

Rapamycin potently inhibits ISR, primarily by reducing neointima formation. Multiple processes contribute to neointima formation after stenting, including cell proliferation, migration, reorganisation of thrombus and extracellular matrix synthesis (reviewed in [32]). Inhibition of ISR is likely to involve mitigation of one or more of these processes. Research into the effects of rapamycin on the vasculature has primarily focused on proliferation of normal VSMCs in vitro or after injury in vivo resulting in a neointima derived from healthy, non-atherosclerotic VSMCs. Critically, recent studies have demonstrated that the biology of human ISR differs in many respects to ISR in animal models and normal human VSMCs. Notably, cell proliferation of VSMCs in ISR lesions is increased [20,21], with significant alterations in cell cycle protein expression including cyclin E [20]. Thus, we have examined the molecular effect of rapamycin on VSMCs derived from established human ISR lesions.

We find that rapamycin potently inhibits human ISR VSMC proliferation. Importantly, high doses of rapamycin do not induce apoptosis, a significant finding if overlapping stents, which result in high drug concentrations at stent locations, are used. Indeed, recent studies indicate that rapamycin may protect against apoptosis [5].

Rapamycin-mediated inhibition of proliferation was only evident in G1 phase in ISR-VSMCs; S-phase or G2/M arrests were not apparent, despite the altered cell cycle regulator expression seen in ISR cells[20]. Although rapamycin profoundly inhibits ISR-VSMCs entering G1 from G0, pRB phosphorylation status was not affected. Moreover, cells expressing E1A remain susceptible to rapamycin-mediated arrest, confirming that pRB is not required for growth arrest by rapamycin. As well as inhibiting pRB, E1A bypasses the effects of elevated p27 expression [29,30]. Therefore, our data suggest that p27 is also not required in rapamycin-mediated arrest of ISR-VSMCs. These results differ from previous publications [7,33] and may reflect the altered expression of cell cycle regulatory proteins in ISR cells compared to their normal counterparts [20]. Indeed, rapamycin had a greater effects on p27 and p27 associated with cyclin D in normal VSMCs, although pRB phosphorylation was still not altered in normal VSMCs. High levels of cyclin E expression, such as those found in ISR VSMCs, are associated with deregulated proliferation, in part by by-passing the pRB/G1 checkpoint [34]. Thus, the anti-proliferative effect of rapamycin on ISR VSMCs is unlikely to be via pRB.

The similarity of response between ISR-VSMC and ISR-DN-p53 demonstrates that p53, a known regulator of G1 and G2 arrest, is also not required for rapamycin to arrest ISR-VSMCs. Moreover, no apoptosis was evident in ISR-DNp53 VSMCs, suggesting that rapamycin will not induce apoptosis in ISR cells in vivo, even if p53 function is compromised.

As rapamycin directly inhibits the function of mTOR, we studied mTOR-dependent pathways in ISR-VSMCs. 4E-BP1 phosphorylation was decreased by rapamycin in ISR-VSMCs and ISR-E1A cells. 4E-BP1 is known to be rate-limiting for G1 progression by preventing the synthesis of ribosomal proteins and may explain the delayed progression through G1 seen in rapamycin-treated ISR cells. Given this result and previous reports of inhibition of matrix production in other models [18] we expected to see a decrease in protein synthesis in rapamycin-treated ISR cells. However, there was no detectable inhibition of protein synthesis, or specifically of collagen production, despite high doses of rapamycin, under conditions where protein synthesis inhibition by cycloheximide could easily be detected. Some studies have suggested that PI3K also regulates this pathway and a concomitant PI3K block may be required to inhibit protein synthesis [15,35]. mTOR also regulates cell size [31,36] and we find that rapamycin inhibits ISR VSMC cell size in post-G1 phases of the cell cycle. As mTOR is also important as a nutrient sensing mechanism [36], the reduction in cell size seen with rapamycin may also demonstrate that cells are exhibiting a starvation phenotype. This reduction in cell size at G2/M may contribute to arrest at the next G1, although transit to G1 is not affected by rapamycin.

p70^{60K} is profoundly inhibited by rapamycin in ISR-VSMCs; indeed, the phosphorylated form is almost completely absent in rapamycin-treated cells, including ISR-E1A cells. Regulation of p70^{60K} is complex, indicative of the burgeoning roles assigned to mTOR, including a critical role in the regulation of proliferation [12]. Interestingly, recent publications suggest that rapamycin may induce delayed cell cycle progression by decreasing the activity of cdk2 [37]. This is particularly relevant in cells expressing high levels of cyclin E, such as ISR-VSMCs [20], which are particularly sensitive to the cdk2 inhibitor Roscovitine [20]. The critical role of cdk2 in proliferation of ISR-VSMCs may further explain the potency of rapamycin on these cells.

In summary, we find that rapamycin is a potent inhibitor of proliferation of ISR VSMCs, despite their previously demonstrated aberrant cell cycle control. The effect of rapamycin on ISR cells does not require pRB, p53 or p27 proteins previously implicated in earlier studies. In contrast, potent effects of rapamycin on p70^{60K} or 4E-BP1 were observed. The growth suppressive effect of rapamycin occurs in G1, but other effects (for example on cell size) occur later and are most likely to be mediated by p70^{60K} or
4E-BP1. Despite a well-recognised effect on cell matrix and collagen synthesis in other systems, rapamycin does not alter either in ISR-VSMCs at concentrations that inhibit proliferation. Our results indicate that proteins such as p70S6K or 4E-BP1 may also represent novel targets for inhibition of ISR.

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References


