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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
G01N 33/563, 33/559, 33/537, 33/531, 33/53, 33/542, C12Q 1/68

A1

(11) International Publication Number: **WO 97/22880**

(43) International Publication Date: 26 June 1997 (26.06.97)

(21) International Application Number: PCT/US96/19671

(22) International Filing Date: 13 December 1996 (13.12.96)

(30) Priority Data:
60/008,801 18 December 1995 (18.12.95) US

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(81) Designated States: AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: USE OF RC-9 IN DIAGNOSIS AND TREATMENT OF PROLIFERATIVE ARTERIAL DISEASE

(57) Abstract

The present invention provides methods of using the polynucleotide sequences, recombinant RC-9 proteins encoded thereby, and RC-9 antibodies in diagnosis and treatment of atherosclerosis.

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USE OF RC-9 IN DIAGNOSIS AND TREATMENT OF PROLIFERATIVE ARTERIAL DISEASE

Field of the Invention

This application relates generally to proteins implicated in coronary artery disease.

Background of the Invention

The use of percutaneous transluminal coronary angioplasty (PTCA) as a treatment for advanced multi-vessel coronary artery disease has increased exponentially in the past decade. However, the long term efficacy of this procedure is significantly limited by the high incidence of vascular restenosis observed in as many as 40% of patients undergoing this procedure [P. Libby *et al.*, Circulation, 86(Suppl. III):47-52 (1992)]. The resultant neointima formation associated with this procedure is a dynamic process actively involving several different cell types and occurring in several phases [A. N. Clowes *et al.*, Lab. Invest., 49:208-215 (1983)]. The initial response is primarily inflammatory in nature involving T-lymphocytes and macrophages which secrete many different cytokines and growth factors seminal to the local inflammatory response. The second phase involves the principle cellular component of the restenotic lesion, the vascular smooth muscle cell (VSMC), which in response the these factors migrates into the intima, secrete soluble growth and chemotactic factors, and proliferate [Libby *et al.*, (1992), cited above; Clowes *et al.*, (1983), cited above; R. Ross, Nature, 362:801-809 (1993); and E. R. O'Brien *et al.*, Circ. Res., 73:223-231 (1993)]. These activated VSMC also secrete extracellular matrix. The third phase may last several weeks post-injury and is characteristic of a chronic fibroproliferative lesion formation [Ross (1993), cited above]. Although the cellular response to balloon angioplasty is fairly well characterized, there is still a lack of effective anti-restenotic pharmacological adjuncts to prevent neointima formation associated with PTCA.

An allograft inhibitory factor (AIF), has been described as useful in diagnosis and treatment of allograft rejection. The sequences of rat and human AIF are provided in WO 95/17506, published June 29, 1995.

What is needed in the art are methods of preventing such neointima formation, as well as better methods of diagnosing and monitoring same.

Summary of the Invention

The present invention provides novel uses for the polynucleotide and amino acid sequences of RC-9, which has been isolated from other cellular materials with which it is naturally associated. Also provided are novel uses for vectors containing these sequences, recombinantly produced RC-9 proteins, and anti-RC-9 antibodies.

Thus, in a first aspect, the present invention provides methods of diagnosing proliferative arterial disease and vascular restenosis. These methods utilize the nucleic acid and amino acid sequences of RC-9 and anti-RC-9 antibodies described herein.

In a further aspect, the present invention provides methods of identifying compounds which specifically bind to a selected RC-9 sequence, including agonists and antagonists, using the RC-9 nucleic acid sequences, proteins, and antibodies described herein. In still a further aspect, the present invention provides for compounds or drugs produced by use of the above methods.

In yet another aspect, the present invention provides methods of treating proliferative arterial disease and vascular restenosis. These methods may utilize the compounds or drugs, nucleic acid and amino acid sequences of RC-9 and, preferably, the anti-RC-9 antibodies described herein.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Drawings

Fig. 1 provides the partial DNA sequences [SEQ ID NO:1] of clone RC9 obtained from differential display. This sequence has been assigned GenBank Accession No. U10894.

Fig. 2 provides genomic DNA sequences [SEQ ID NO:2] of RC-9. This sequence has been assigned GenBank Accession No. U33471.

Fig. 3 illustrates the cDNA [SEQ ID NO:3] and putative amino acid sequences [SEQ ID NO:4] of rat RC-9.

Fig. 4 illustrates the cDNA [SEQ ID NO:5] and amino acid sequences [SEQ ID NO:6] of human RC-9.

Detailed Description of the Invention

The present invention provides novel uses for a gene, termed RC-9, and the encoded protein, in detecting and treating proliferative arterial disease and vascular restenosis. The

nucleotide and amino acid sequences and antibodies provided herein are useful in diagnosis and monitoring such vascular disease and damage. These sequences and antibodies are further useful in generating compounds useful in treatment of atherosclerosis and other conditions characterized by vascular stenosis.

The observation of RC-9 DNA cross-hybridization with DNA from a variety of animal species suggests substantial evolutionary conservation and a potentially important biologic function for the RC-9 encoded protein. The temporal expression of RC-9 mRNA in balloon angioplasty injured rat carotid artery indicates that its protein product plays a role in the early to middle stages of vascular restenosis.

I. RC-9 Polynucleotide Sequences

The methods of the invention may utilize the RC-9 polynucleotide sequences described herein. Figs. 1-4 provide DNA sequences of the novel transcript of the invention. Fig. 1 provides the nucleotide sequences of clone RC9 [SEQ ID NO:1], obtained as described in Example 1 below. This partial sequence is 424 bases in length and was used as a probe to obtain the longer sequence of Fig. 2. Fig. 2 provides genomic DNA sequences of RC-9 [SEQ ID NO:2], including two introns. Intron 1 spans base pairs 121-772 and intron 2 spans base pairs 1000-1276. PolyC tracts are located at base pairs 864-873 and 1254-1276. Branch point consensus sequences are located at base pairs 851-857 and 1212-1218 of Fig. 2. Fig. 3 provides the cDNA sequences of rat RC-9 [SEQ ID NO:3]. The transcription initiation codon is located at bp 143-145 and the stop codon is located at bp 581-583 of Fig. 3. Fig. 4 provides the 636 bp DNA sequences of human RC-9 [SEQ ID NO:5]. These sequences include a 444 bp open reading frame (bp 72-516), which encodes a 147 amino acid protein [SEQ ID NO:6].

Fragments of these sequences may prove useful for a variety of uses. Desirably, these functional fragments are at least about 15 nucleotides in length and encode a desired amino acid sequence, e.g. an epitope, a therapeutically useful peptide desirably characterized by RC-9-like biological activity, or the like. These RC-9 nucleotide sequences may be isolated by conventional uses of polymerase chain reaction or cloning techniques. Alternatively, these sequences may be constructed using conventional genetic engineering or chemical synthesis techniques.

For use in the methods of the invention, the genomic RC-9 DNA sequence (Fig. 1 SEQ ID NO:1 and Fig. 2 SEQ ID NO:2) or a cDNA sequence (Fig. 3 SEQ ID NO:3 and Fig.

4 SEQ ID NO:5) coding for the encoded RC-9 protein or a functional fragment thereof, may be modified. Utilizing the sequence data provided herein, it is within the skill of the art to obtain other polynucleotide sequences encoding the RC-9 proteins useful in the invention. Such modifications at the nucleic acid level include, for example, modifications to the nucleotide sequences which are silent or which change the amino acids, e.g. to improve expression or secretion.

In still another alternative, the polynucleotide sequences useful herein may be modified by adding readily assayable tags to facilitate quantitation, where desirable. Nucleotides may be substituted, inserted, or deleted by known techniques, including, for example, *in vitro* mutagenesis and primer repair. Also included are allelic variations, caused by the natural degeneracy of the genetic code.

In addition to isolated nucleic acid sequences encoding the RC-9 protein described herein, this invention also encompasses other nucleic acid sequences, including those complementary to the illustrated DNA sequences, such as antisense sequences. Useful DNA sequences also include those sequences which hybridize under high or moderately high stringency conditions [see, T. Maniatis *et al.*, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences illustrated in Figs. 1-4 [SEQ ID NO:1, 2, 3, 5]. An example of a highly stringent hybridization condition is hybridization at 4XSSC at 65°C, followed by a washing in 0.1XSSC at 65°C for an hour. Alternatively, an exemplary highly stringent hybridization condition is in 50% formamide, 4XSSC at 42°C. Other, moderately high stringency conditions may also prove useful, e.g. hybridization in 4XSSC at 55°C, followed by washing in 0.1XSSC at 37°C for an hour. Alternatively, an exemplary moderately high stringency hybridization condition is in 50% formamide, 4XSSC at 30°C.

As described in more detail below, the RC-9 nucleic acid sequences encoding these proteins are useful for a variety of diagnostic and therapeutic uses. Advantageously, the nucleic acid sequences are useful as diagnostic probes and antisense probes for use in the detection and diagnosis of proliferative arterial disease and vascular restenosis, among other conditions associated with undesirable RC-9 levels or expression. Oligonucleotide probes may be useful in such standard diagnostic techniques as Southern blotting and polymerase chain reaction.

Alternatively, the RC-9 nucleic acid sequences may be used to produce RC-9 proteins useful in the methods of the invention. Once constructed, or isolated, these DNA

sequences or suitable fragments are preferably employed to obtain proteins of this invention, *in vitro* or *in vivo*.

II. RC-9 Amino Acid Sequences

The methods of the invention may utilize RC-9 amino acid sequences, including the RC-9 proteins provided herein and suitable functional fragments thereof. The RC-9 rat protein [SEQ ID NO:4] is encoded by the cDNA sequences [SEQ ID NO:3] illustrated in Fig. 3 and the RC-9 human protein [SEQ ID NO:6] is encoded by the cDNA sequences of Fig 4 [SEQ ID NO:5]. The rat protein [SEQ ID NO:4] is 147 amino acids in length and has a molecular weight of 16824.9 Daltons. The human protein is also 147 amino acids in length [SEQ ID NO:6]. Also useful in the methods of the invention are biologically active fragments of RC-9. These functional fragments are desirably at least five amino acids in length and may encompass an epitope or other desired amino acid sequence.

Also useful in invention are analogs, or modified versions, of the RC-9 protein. Typically, such analogs differ by only 1, 2, 3 or 4 codon changes and are characterized by RC-9-like biological activity. Examples include polypeptides with minor amino acid variations from the illustrated amino acid sequences of RC-9 (Figs. 3 and 4, SEQ ID NOs: 4 and 6); in particular, conservative amino acid replacements. Conservative replacements are those that take place within a family of amino acids that are related in their side chains and chemical properties. Additionally, the RC-9 proteins useful in the invention may be modified, for example, to improve production thereof, to enhance protein stability or other characteristics, e.g. binding activity or bioavailability, to enhance its use for screening competitive compounds or to confer some other desired property upon the protein.

The RC-9 protein and RC-9 protein fragments described herein are useful in therapeutic compositions, as described in more detail below. The methods of the invention also include the use of these proteins in diagnostic applications, as well as for generation of other therapeutic and diagnostic reagents, such as anti-RC-9 antibodies. In common with other proteins generally, these RC-9 proteins may also serve in screening assays or as research tools. More desirably, the RC-9 proteins are also useful for the screening and development of chemical therapeutic agents useful for preventing the action of RC-9, e.g., in restenosis.

III. Recombinant Expression of RC-9

The DNA sequences described herein may be used to produce recombinant RC-9 proteins. The resulting proteins may be used in the methods of the invention, or the method of the invention may involve *in vivo* expression of the proteins. To produce the RC-9 proteins, the RC-9 DNA sequences may be inserted into a suitable expression system. Desirably, a recombinant molecule or vector is constructed in which the cDNA encoding RC-9 is operably linked to a heterologous expression control sequence permitting expression of the RC-9 protein. Numerous types of appropriate expression vectors and host cell systems are known in the art for mammalian (including human) expression, insect, e.g., baculovirus expression, yeast, fungal, and bacterial expression, by standard molecular biology techniques.

The transformation of these vectors into appropriate host cells can result in expression of the selected RC-9 proteins. Other appropriate expression vectors, of which numerous types are known in the art, can also be used for this purpose.

Suitable host cells or cell lines for transfection by this method include insect cells, such as *Spodoptera frugipedera* (Sf9) cells. Methods for the construction and transformation of such host cells are well-known. [See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein].

Similarly, mammalian cells, such as Human 293 cells, rat aortic vascular cell lines [E. H. Ohlstein *et al.*, Eur. J. Pharmacol. - Mol. Pharmacol. Section, 225:347-350 (1992)], Chinese hamster ovary cells (CHO), the monkey COS-1 cell line or murine 3T3 cells derived from Swiss, Balb-c or NIH mice may be used. Suitable mammalian host cells and methods for transformation, culture, amplification, screening, production and purification are known in the art. [See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman *et al.*, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley *et al.*, U. S. Patent 4,419,446]. Another suitable mammalian cell line is the CV-1 cell line.

Similarly useful as host cells are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, MC1061, and strains used in the following examples) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas*, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides useful in the methods of the invention. Other fungal cells may also be employed as expression systems.

Thus, the present invention provides a method for producing a recombinant RC-9 protein which involves transforming a host cell with at least one expression vector containing a recombinant polynucleotide encoding a RC-9 protein under the control of a transcriptional regulatory sequence, e.g., by conventional means such as transfection or electroporations. The transformed host cell is then cultured under suitable conditions that allow expression of the RC-9 protein. For example, host cells, e.g., rat aortic vascular smooth muscle cells, may be transfected with sufficient vectors that they are capable of overexpressing the RC-9 protein, making them useful for screening compounds which inhibit RC-9 expression. In another embodiment, the expressed protein is recovered, isolated, and purified from the culture medium (or from the cell, if expressed intracellularly) by appropriate means known to one of skill in the art.

For example, the proteins may be isolated following cell lysis in soluble form, or extracted in guanidine chloride. If desired, the RC-9 proteins of the invention may be produced as a fusion protein. For example, it may be desirable to produce such RC-9 fusion proteins, to enhance expression of the protein in a selected host cell, or to improve purification. Suitable fusion partners for the RC-9 proteins described herein are well known to those of skill in the art and include, among others, b-galactosidase and poly-histidine.

IV. Production of Anti-RC-9 Antibodies

The RC-9 proteins, as well as modified versions or analogs thereof, or cells expressing same, are useful as antigens for the development of antibodies to RC-9. Antibodies useful in the methods of this invention include monoclonal, polyclonal, chimeric, single chain and humanized antibodies, as well as Fab fragments. These antibodies may be produced by conventional methods, including the Kohler and Milstein hybridoma technique, recombinant techniques, such as described by Huse *et al.*, Science, 246:1275-1281 (1988), or any other modifications thereof known to the art. Techniques described for the production of single chain antibodies (US Patent No. 4,946,778) can be adapted to produce single chain antibodies to the RC-9 proteins descried herein. Also, transgenic mice, or other organisms such as mammals, may be used to express humanized antibodies to a RC-9 protein or RC-9-derived protein. The antibodies of this invention may themselves be used to generate anti-idiotype antibodies. Techniques for generating such antibodies are well-known in the art.

The antibodies of the invention may be utilized in protein form. Alternatively, the antibodies of the invention may be utilized in the form of a polynucleotide, which expresses the antibody or a functional fragment thereof (e.g., a single chain or a Fab fragment) in vivo.

V. Diagnostic Reagents

The RC-9 proteins, antibodies, and polynucleotide sequences (including anti-sense polynucleotide sequences) described herein may be used as diagnostic reagents for diagnosing certain vascular disorders, e.g., atherosclerosis, associated with production or excessive production of RC-9. For example, a RC-9 protein, antibody, or polynucleotide may be utilized to diagnose vascular damage characteristic of such a condition. These reagents may optionally be labelled using diagnostic labels, such as radioactive labels, colorimetric enzyme label systems and the like conventionally used in diagnostic or therapeutic methods. The reagents may measure RC-9 levels in selected mammalian tissue in conventional diagnostic assays, e.g., Southern blotting, Northern and Western blotting, polymerase chain reaction and the like. For example, as diagnostic agents the polynucleotide sequences may be employed to detect or quantitate normal RC-9 mRNA or detect mutations in target gene RNA in a patient sample. Such a method may utilize PCR primers complementary to the nucleic acid sequence of Figs. 1-4 [SEQ ID NO:1, 2, 3, 5]. Alternatively, the detection of a specific DNA sequence (i.e., RC-9) may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, e.g., restriction fragment length polymorphisms (RFLP) and Southern blotting of genomic DNA. Other suitable assays utilize a RC-9 protein, protein fragment, or anti-RC-9 antibody as a reagent. These assays include radioimmunoassays, competitive-binding assays, Western blot analysis and ELISA assays. The selection of the appropriate assay format and label system is within the skill of the art and may readily be chosen without requiring additional explanation by resort to the wealth of art in the diagnostic area.

Thus, the present invention provides methods for the use of these RC-9 protein, antibody or polynucleotide reagents in the diagnosis of disorders characterized by vascular restenosis, such as atherosclerosis. The methods may involve contacting a selected sample, e.g., blood, plasma, serum, or other suitable cells with the selected reagent, protein, antibody or DNA sequence, and measuring or detecting the amount of RC-9 present in the sample in a selected assay format based on the binding or hybridization or the reagent to the sample.

VI. Therapeutic Reagents

The invention further provides methods for treatment of vascular restenosis. More particularly, this method involves administration of an anti-RC-9 antibody for blocking RC-9 activity in mammalian tissue. Also useful as therapeutic reagents of this invention are anti-idiotype antibodies, which can be used to block binding of RC-9 to its corresponding receptor. Although less desired, the therapeutic reagent may be a RC-9 nucleic acid sequence, a vector containing the nucleic acid sequences or an RC-9 protein. Alternatively, the therapeutic reagent may be a drug obtained using the methods described below.

As one example, the therapeutic reagents may be administered by appropriate routes in a pharmaceutical composition. Generally, the composition contains between about 10 mg to about 10 mg of the active agent (e.g., anti-RC-9 antibody, or a vector or polynucleotide described herein) per kg body weight. It will be appreciated that optimum dosage may be adjusted by one skilled in the art, taking into account the indication, its severity, route of administration, and the like.

Suitable pharmaceutical carriers are well known to those of skill in the art and can be readily selected from among saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof, among others. The pharmaceutical compositions of the invention may optionally contain other active ingredients, or other desirable components, e.g., pH adjusters, preservatives, and the like.

Appropriate routes may be readily determined by one of skill in the art and include, for example, intravenous, intramuscular, subcutaneous, intraperitoneal, interdermal, oral, vaginal, anal, intranasal, and topical routes. Currently, the preferred method of administration is intravenous. However, one of skill in the art can readily select another appropriate route of administration. Dosing may be repeated as needed or desired.

VII. Drug Screening and Development

The present invention also provides methods using the RC-9 proteins, antibodies and polynucleotide sequences described herein in the screening and development of chemical compounds or proteins which have utility as therapeutic drugs for the treatment of atherosclerosis and other vascular disorders.

As one example, such a compound is capable of binding to RC-9 and either enhancing (act as an agonist) or blocking (act as an antagonist) its biological activity. Such compounds are anticipated to be useful as a drug component for the treatment or prevention

of vascular disorders, as described above. Presently, conventional assays and techniques exist for the screening and development of drugs capable of competitively binding to selected regions of RC-9. These include the use of phage display system for expressing the RC-9 proteins or portions thereof, and using a culture of transfected *E. coli* or another microorganism to produce the proteins for binding studies of potential binding compounds. See, for example, the techniques described in G. Cesarini, FEBS Letters, 307(1):66-70 (July 1992); H. Gram *et al.*, J. Immunol. Meth., 161:169-176 (1993); C. Summer *et al.*, Proc. Natl. Acad. Sci., USA, 89:3756-3760 (May 1992), incorporated by reference herein.

Other conventional drug screening techniques may be employed using the proteins, antibodies or polynucleotide sequences useful in this aspect of the invention. As one example, a method for identifying compounds which specifically bind to RC-9 DNA sequences can include simply the steps of contacting a selected RC-9 DNA fragment with a test compound to permit binding of the test compound to the DNA fragment; and determining the amount of test compound, if any, which is bound to the DNA fragment. Such a method may involve the incubation of the test compound and the RC-9 DNA fragment immobilized on a solid support.

Another method of identifying compounds which specifically bind to RC-9 sequences can include the steps of contacting a RC-9 DNA fragment immobilized on a solid support with both a test compound and the protein sequence which is a receptor for RC-9 to permit binding of the receptor to the RC-9 DNA fragment; and determining the amount of the receptor which is bound to the DNA fragment. The inhibition of binding of the normal protein by the test compound thereby indicates binding of the test compound to the RC-9.

RC-9-like effects of potential agonists, or the effects of potential antagonists, may be measured, for instance, by determining activity of a reporter system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of RC-9 or molecules that are found to elicit the same effects as RC-9. Reporter systems that may be useful in this regard include but are not limited to colorimetric labeled substrate converted into product, a reporter gene that is responsive to changes in RC-9 activity and binding assays known in the art. Other suitable methods are well known to those of skill in the art.

Thus, through use of such methods, the present invention provides compounds capable of interacting with RC-9 or portions thereof, and either enhancing or decreasing its biological activity, as desired. Such compounds are encompassed by this invention.

The following examples which disclose the cloning and expression of RC-9 are for illustrative purposes only, and should not be construed as limiting this invention in any way.

Example 1 - Isolation of RC-9

A. Rat Left Common Artery Balloon Angioplasty

Left common carotid artery balloon angioplasty was performed under aseptic conditions on male Sprague-Dawley rats [350 g; Charles River Breeding Laboratory Inc., Wilmington, MA] under sodium pentobarbital anesthesia [65 mg/kg, i.p.- Steris Laboratories, Phoenix, AZ] as described previously [E. H. Ohlstein *et al.*, Proc Natl Acad Sci USA, 90:6189-6193 (1993)]. Briefly, the left external carotid artery was identified and cleared of adherent tissue, allowing the insertion of a 2-F Fogarty arterial embolectomy catheter [Model 12-060-2F; Baxter Healthcare, Santa Ana, CA)]. The catheter was guided a fixed distance down the common carotid artery to the aortic arch, inflated with a fixed volume of fluid, and withdrawn back to the site of insertion. This procedure was performed a total of three times. Once completed, the catheter was removed, and the wound was closed (9-mm Autoclips; Clay Adams, Franklin Lakes, NJ) and swabbed with Povadyne surgical scrub (7.5% Povidone-Iodine- Chaston Dayville, CT). Animals were housed in Plexiglass cages under a 12-hour light\dark cycle with access to standard laboratory chow and drinking water ad libitum until required for tissue collection.

To isolate the carotid arteries, rats were exsanguinated via the vena cava under barbiturate anesthesia (100 mg/kg, i.p.). Left common carotid arteries were rapidly cleared of adherent tissue in situ, isolated, and placed directly in guanidine thiocyanate (Promega Company, Madison, WI). These vessels were then immediately flash frozen in liquid N_2 and stored at -80°C until required for RNA isolation. For subsequent Northern analysis, tissues were examined from naive animals (control) and from animals that had undergone angioplasty 6 hours, 3 days, and 14 days before to study the differential expression of transcripts in a more detailed, temporal fashion. Northern analysis was also performed on sham vessels.

B. RNA isolation and Northern analysis

For each time point studied, four or five left carotid arteries were pooled, or VSMC from culture isolated and total RNA obtained as described [P. Chomczynski and N. Sacchi, Ann. Biochem., 162:156-159 (1987)]. Equal amounts of RNA were loaded and separated on a 1.3% agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized

(0.25M NaCl, 1% sodium dodecyl sulfite, 50% formamide, 2X Denhardt's solution, 25mg denatured salmon sperm DNA, 5% dextran sulphate 42°C overnight) with the indicated probe. Blots were washed under high stringency (0.1% sodium citrate, 0.1% sodium dodecyl sulfate, 65°C), and exposed to film for 6 - 48 hr. at -80°C. All probes were [$a^{32}P$]-labeled by the random priming method (Boehringer Mannheim, Indianapolis, In.) (all isotopes were from Amersham Inc., Arlington Heights IL.). The same filter was stripped and subsequently hybridized with the various DNA probes. The glyceraldehyde-3-phosphate dehydrogenase (G3PDH) probe was generated from PCR amplimers (Clonetech, Palo Alto, CA.). Relative intensities of hybridization signals were obtained by densiometric scanning (RFLP-Scan Software, Scanalytics, Inc.) of autoradiograms exposed within the linear range of the film (Kodak X-OMAT). Human and multiple tissue Northern blots were purchased from Clonetech, Inc. (Palo Alto, CA).

C. Differential Display of mRNA

Total RNA from control left common carotid arteries and those that had undergone balloon angioplasty 3 and 14 days earlier were isolated, and the differential display technique was used to examine changes in gene expression. DNA was removed from total RNA using a sequential procedure of DNA digestion, (1 U DNase; Gibco-BRL, Gaithersberg, MD), phenol/chloroform extraction, and ethanol precipitation according to the manufacturer's protocol. Purified total RNA (0.2 mg) was reverse transcribed using modified oligo dT primers (reverse transcriptase from GenHunter Corporation, Brookline, MA) [P. Liang *et al.*, Science, 257:967-971 (1992)]. cDNA was amplified and labeled by including 10% of the reverse transcription reaction with the appropriate 3' oligo dT primer, dNTPs, [^{35}S]dATP, and an arbitrary 10-mer 5' primer (GenHunter). Forty PCR cycles were run with the following parameters: 94°C for 30 seconds; 40°C for 120 seconds; 72°C for 45 seconds, with the last cycle followed by a 72°C soak for 7 minutes. The labeled cDNAs obtained from this reaction were separated on a 6% acrylamide sequencing gel, which was dried and exposed to autoradiographic film 12 to 48 hours.

Gene expression in day 3 vessels represents early molecular events in the pathogenesis of lesion formation (i.e., those events occurring before the physical formation of a neointimal lesion), whereas day 14 vessels represent later events (i.e., when approximately 80% of the final lesion volume has formed in the neointima) [S. A. Douglas *et al.*, Eur. J. Pharmacol., 255:81-89 (1994)].

For this study, RNA from these samples was reverse transcribed using all four oligo dT anchor primers, and the resulting cDNA was amplified using two different 5' decamers. Attention was paid primarily to bands in the top third of the sequencing gel, because these samples are longer and therefore facilitate DNA sequence identification. Focusing on the top third of the sequencing gel, less than 10% of the bands appeared to be differentially expressed (8 of 82). In this initial study, two bands were selected that displayed differential expression.

D. Recovery Reamplification and Cloning of cDNAs

Selected cDNAs were excised from the sequencing gel, eluted, and reamplified using the same primers as previously described [Liang *et al.*, cited above]. The reamplified PCR product was visualized on 2.0% agarose gel, stained with ethidium bromide, and recovered by glass extraction (Bio 101, La Jolla, CA). Reamplified PCR products were labeled as probes as described above and were cloned into the pCRII vector using the Invitrogen TA cloning system (San Diego, CA), and DNA was sequenced on an applied biosystems model 373A automated sequencing apparatus.

The DNA segments were recovered from the acrylamide sequencing gel by excision and elution and PCR amplified using the same primers and reaction conditions. Reamplification yielded single products, the size of which are consistent with their apparent sizes displayed on the acrylamide sequencing gel (data not shown). These reamplification products were then glass-purified from the gel and used as probes for Northern analysis.

Only two of the rat tissues examined expressed the RC9 transcript. Testes RNA showed a strong signal of approximately 1.35 kb, slightly larger than the 1.1 kb observed in carotid arteries. A second, much weaker signal of approximately 1.1 kb was detected in spleen. In addition to providing information on which cDNA library to choose, this data indicates that the expression of this transcript is tissue specific, and the differences in size from testes to spleen suggest processing of this mRNA transcript, or the presence of similar, messages in different tissues.

Total RNA from naive rat carotid arteries and from carotid arteries isolated at three timepoints after balloon angioplasty were probed with these DNA fragments in Northern analysis to confirm their differential expression. Each of these DNAs was found to represent a discrete gene transcript. Northern analysis verified their expression pattern. RC9 expression is undetectable in naive vessels, is detected at low levels 6 hours postsurgery, reaches maximal levels at day 3, and declines in day 14 vessels, confirming the pattern

observed in the differential display gel. No expression of RC9 mRNA is detected in sham control vessels.

Each of these cDNAs were subcloned into the pCRII vector and dideoxy sequenced in both directions. The RC9 clone is 424 bases (Fig. 1, SEQ ID NO:1) and displays no significant sequence homology to any previously characterized genes deposited in Genbank.

Example 2 - Isolation of RC-9

RC9 is a 424 bp partial genomic DNA fragment [SEQ ID NO:1] isolated from differential display analysis of rat carotid arteries post-balloon angioplasty, and identifies an mRNA of approximately 1.1 kb, as described in Example 1 above. To fully identify and characterize this transcript, it was essential to obtain the full length gene.

Since cDNA libraries prepared from restenotic rat carotid arteries are unavailable, it was necessary to identify a rat tissue in which this message was expressed and then screen a cDNA library prepared from mRNA from this tissue.

A. cDNA library screening

A rat testes cDNA library (Stratagene, Inc.) was screened at high stringency (0.1X SSPE at 65°C) with the 424 bp RC9 [SEQ ID NO:1]. This probe was used to screen approximately 72,000 plaques, of which three were positive after three rounds of screening. These three clones, each containing an insert of approximately 1.68 kb flanked by EcoRI sites as part of the vector polylinker were obtained and shown to be identical by restriction analysis. One clone was chosen and shown to be 98% homologous to RC9 by dideoxy nucleotide sequencing using an RC9 specific primer and also used to probe rat carotid arteries subject to balloon angioplasty to confirm the identity of this clone by mRNA expression.

Total RNA from naive rat carotid arteries and from carotid arteries isolated at three timepoints (1, 3 and 7 days) following balloon angioplasty were size fractionated and hybridized with a radiolabeled PCR product representing the insert of RC-9. More specifically, total RNA (10 mg) was separated on a 1.2% agarose/formaldehyde gel, transferred to nitrocellulose, hybridized, and washed as described above. A glyceraldehyde-3-phosphate dehydrogenase (G3PDH) probe was used as a loading control. Scanning densiometric analysis of this blot normalized to glyceraldehyde- 3-phosphate dehydrogenase (G3PDH) content confirms a 9-fold increase in expression of this message

over basal levels 1 day post balloon angioplasty, 4-fold 3 days, and 2-fold 7 days post-injury. This expression pattern consistent with that observed for the RC9 differential display fragment, with the exception of a basal level detected in naive vessels, and indicates that similar to expression of this gene is induced in rat carotid arteries in response to balloon angioplasty. Northern analysis of RC-9-1 mRNA from sham control vessels shows no change in basal expression (data not shown).

B. Sequence Analysis

The clone was sequenced on both strands in its entirety (Sequenase, United States Biochemical Corporation) as previously described. DNA and protein sequences were analyzed using the Mac Vector software package (International Biotechnologies, Inc.). Searches for sequence similarity were performed using the GenBank Nucleic Acid Database and the Genetics Computer Group program.

The 3' region of the 1.68 kb insert from this clone was identified as 98% homologous with the 424 bp fragment obtained from differential display, further confirming the identity of this clone as the sequence of RC9. The nucleic acid clone RC-9-1 is shown in Figure 2, SEQ ID NO:2. Subsequent analysis of the sequences of Fig. 2 [SEQ ID NO:2] revealed the presence of polyC tracts at base pairs 864-873 and 1254-1276 and branch point consensus sequences at base pairs 851-857 and 1212-1218. This indicated that the sequence encompassed intronic sequences. Further analysis identified two introns. Intron 1 spans base paris 121-772 of Fig. 2 and intron 2 spans based pairs 1000-1276. The sequences of Fig. 2 [SEQ ID NO:2] were used to obtain a RC-9 cDNA clone, as described in Example 3 below.

C. Genomic DNA species analysis

To determine the evolutionary conservation of the RC-9 sequence of Fig. 2 SEQ ID NO:2, Southern blot analysis was carried out using EcoRI digested genomic DNA from a variety of animal species including human, monkey, rat, mouse, dog, cow, rabbit, chicken, and yeast (*Saccharomyces cerevisiae*). Genomic DNA Southern blots containing 4 mg of genomic DNA from nine different animal species digested was purchased from Clonetech, Inc. (Palo Alto, CA) and was hybridized at high stringency (5X sodium citrate, 10% Denhardt's solution, 100 mg/ml sheared salmon sperm DNA, 1% sodium dodecyl sulfate, 65°C, overnight) with the full-length RC-9 1 cDNA clone according to manufacturers instructions. Blots were washed under low (2.0X sodium nitrate, 0.1% sodium dodecyl sulfate, 55°C), or high stringency (0.2X sodium citrate, 1% sodium dodecyl sulfate, 65°C), and audioradiographed.

The RC-9-1 DNA of Fig. 2 [SEQ ID NO:2] hybridized to number of restriction fragments from various species under low stringency washing conditions (2X SSC, 0.1% SDS, 50°C), the most prominent being a 24 kb fragment in both rat and cow, and a 4.4 kb fragment in mouse. Other bands were detectable in dog, mouse, rat, and cow. No bands were visible in rabbit, chicken, or yeast. A number of less prominent bands were also observable at this stringency, including a band approximately 2.3 and 1.0 kb in length visible in human, rat, and cow. A strong signal at 2.5 kb in mouse is also prominent. Under higher stringency washing conditions, (0.2 X SSC, 1% SDS, 65°C), these bands are barely visible, and the remaining prominent bands of 24 kb in rat and 4.4 kb in mouse did not change in intensity. The 2.5 kb band in mouse is no longer seen, suggesting that this band represents another gene that cross-hybridizes with RC-9-1 sequences of Fig. 2 [SEQ ID NO:2]. Thus it appears that the RC-9-1 transcript is well conserved.

Example 3 - cDNA Cloning of RC-9

Rat tested mRNA was isolated as previously described and reverse transcribed to yield cDNA using oligo-dT primer. RC-9 primers were chosen from the extreme ends of the RC-9 genomic DNA (Fig. 2, SEQ ID NO:2). More particularly, the sense primer (25 mer oligonucleotide) corresponds to nt 31- nt 55 of Fig. 2 [SEQ ID NO:2] and has a sequence of 5' - ACT TCA GAC TCT CTC TTC CCT ACG G - 3'; the anti-sense primer (28 mer oligonucleotide) corresponds to nt 1651-1678 of Fig. 2 [SEQ ID NO:2] and has a sequence of 5' - GCA TCA GGG AGC ATT ATT TAT TTA GTT T - 3'. Polymerase chain reaction (PCR) was used to obtain the RC-9 cDNA. Forty PCR cycles were run using the following parameters: 94°C for 1 min; 53°C for 1 min; 72°C for 3 min, with the last cycle followed by a 4°C soak. The RC-9 cDNA was separated on a 1% agarose gel and purified using standard techniques. The RC-9 cDNA was ligated into the vector pCRII [InVitrogen], clones were isolated and restriction enzyme digested with EcoRI to confirm they contained an insert of the correct size. Positive clones were sequenced and compared to the genomic sequence of Fig. 2. A partial cDNA sequence is provided in Fig. 3 [SEQ ID NO:3], together with the amino acid sequence encoded thereby. The sequence of Fig. 3 is 696 basepairs in length [SEQ ID NO: 3]. A homology study performed with a cDNA sequence corresponding to nucleotides 72-696 of Fig. 3 revealed the cDNA sequence [SEQ ID NO:3] was 98% homologous to the RC-9 genomic sequence of Fig. 2 [SEQ ID NO:2].

SEQ ID NO:3 and 4 have been found to have substantial identity to the rat AIF sequences, previously described in WO 95/17506.

Example 4 - Function of RC-9

The RC-9 clone of Example 3 was cut out of the pCRII plasmid using the restriction endonuclease BstX1. The RC-9 DNA was purified and ligated into the expression vector pRc/CMV (Invitrogen Inc.) which had been pre-cut with BstX1 and grown in *E. coli*. A single bacterial colony was chosen containing RC-9 in the correct orientation for propagation. Purified DNA was obtained with RC-9 in pRc/CMV.

Rat aortic vascular smooth muscle (RAVSM) cells were transfected with either pRc/CMV plasmid alone or with plasmid pRc/CMV containing RC-9. Transfection was done using LipofectAMINE Reagent (Life Technologies Inc.) mixed with the DNA to be transfected (pRc/CMV or pRc/CMV RC-9). The LipofectAMINE Reagent allows the DNA to pass through the cell membrane, eventually permitting the DNA to be incorporated into the host's chromosomes. Two days following transfection, the compound G418 (Geneticin) was added to the cells. G418 kills cells that do not contain the pRc/CMV plasmid, leaving only cells containing pRc/CMV or pRc/CMV RC-9. Transfected RAVSM were grown in the presence of G418 for functional studies on RC-9.

RAVSM containing either pRc/CMV or pRc/CMV RC-9 were seeded onto T-150 flasks at equal densities and left to grow over a period of 5 or 7 days in DMEM + 10% FBS + G418. The cells were then trypsinized and counted using a hemocytometer to look for differences in proliferation. At 5 days, cell counts for pRc/CMV were $4.55 \times 10^4 \pm 8.5 \times 10^3$ cells/cm^2 (in a T-150 flask @ 150 cm^2) and for pRc/CMV RC-9 $1.22 \times 10^5 \pm 5 \times 10^2$ cells/cm^2; a change of 168%. The 7 day cell counts yielded similar results: cell counts for pRc/CMV were $5.93 \times 10^4 \pm 5.5 \times 10^3$ cells/cm^2 (in a T-150 flask @ 150 cm^2) and for pRc/CMV RC-9 $1.84 \times 10^5 \pm 3.7 \times 10^4$ cells/cm^2; a change of 210%.

The cell count data clearly shows that RAVSM cells containing RC-9 proliferate at a higher rate than control cells.

Example 5 - Tissue Expression of Human RC-9

The human RC-9 sequences described herein, SEQ ID NO:5 and 6, have substantial identity to the human AIF sequences described previously WO 95/17506. *E. coli*

transformed with human RC-9 [SEQ ID NO:5], which was originally expressed in pBluescript, were grown and positive clones identified.

Gel purified cDNA probe (generated by PCR), was used to assess human RC-9 mRNA expression by Northern "dot blot" analysis, as described above. Expression, normalized using cyclophilin, was relatively low (defined by hRC-9:cyclophilin ratios of < 1.3) in "normal" human tissue from various regions of the CNS, GI/urogenital tract and, surprisingly, heart, aorta and testes. (This last observation is particularly interesting since rat RC-9 [SEQ ID NO:3] was cloned from a rat testes cDNA library). Expression was, however, elevated several-fold in organs associated with inflammatory cell function (bone marrow > thymus > spleen > peripheral blood leukocytes, e.g., hRC-9:cyclophilin ratios of >6.0). Fetal tissue showed a similar pattern of expression (e.g., highest expression in thymus, spleen). In human "diseased" tissue blots, enhanced mRNA was present in mitogen (TGFb or angiotensin II) stimulated vascular smooth muscle cells demonstrating upregulation of human RC-9 [SEQ ID NO:6] in association with growth factor stimuli. Tissue analysis demonstrated elevated human RC-9 hybridization (e.g., in hearts and kidneys) in association with hypertension, congestive heart failure and myocardial infarction consistent with a role of human RC-9 [SEQ ID NO:5 and 6] in the control of cellular proliferation/differentiation and the pathogenesis of fibroproliferative disorders. In accord, relative to medial expression, human RC-9 mRNA expression was also augmented in vascular smooth muscle cells derived from the tunica neointima of blood vessels which had previously undergone angioplasty.

All references cited herein are incorporated by reference. While certain embodiments of the invention have been particularly described, it will be apparent to those skilled in the art that many modifications and variations may be made. Therefore, the present invention is not to be construed as limited by any of the particular embodiments shown, rather its scope will be defined only by the claims which follow.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SmithKline Beecham Corporation
Ohlstein, Eliot
Arleth, Anthony
Autieri, Michael V.

(ii) TITLE OF INVENTION: Use of RC-9 in Diagnosis and Treatment of Proliferative Arterial Disease

(iii) NUMBER OF SEQUENCES: 8

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: WO
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/008,801
(B) FILING DATE: 18-DEC-1995

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 33,777
(C) REFERENCE/DOCKET NUMBER: P50420-1

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 610-270-5090

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 424 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCCAGCGAA GTACATGGAG TTTGATCTGA ATGGCAATGG AGATATCGAT ATTATGTCCT 60

TGAAGCGAAT GCTGGAGAAA CTTGGGGTTC CCAAGACCCA TCTAGAGCTG AAGAAATTAA 120

TTAGAGAGTT GTCCAGTGGC TCCGAGGAGA CGTTCAGTTA CTCTGACTTT CTCAGAATGA 180

TGCTGGGCAA GAGATCTGCC ATCTTGAGAA TGATTCTGAT GTATGAGGAG AAAAACAAAG 240

AACACCAGAA GCCAACTGGT CCCCCAGCCA AGAAAGCTAT TTCTGAGTTG GCCTAATGGA 300

GGTGGATATA ACACGGTGGG ACCGAGGACC TTCGTGTCAG CAGCATGGGA AGAGAAGAAT 360

CAGTTGCGAG CCAGACTGTC AATGCTCAAT AAATAATGCT CCCTAGTGCC TGAAAAAAAA 420

AAAA 424

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1678 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTCTCACCCC ATTCTTGGAG CAGCCTGCAG ACTTCAGACT CTCTCTTCCC TACGGAGAAA 60

GTCAGCCAGT CCTCCTCAGC TGCTTCTTAA CCTGCACCAT GAAGCCTGAG GAAATTTCAA 120

GTAACCCTCC CAGCCCCCAC CCCAGGGTCT GGGGAAGAAG TCATTTTTGT GTTTCCTTGG 180

TTTGGGTTTG AGACAGGTTC TTACTGTGTA GGCCTGGCTG GTCTCCAACT CCCAGGATCC 240

TCTAGCCTCA ACCTCTCTTA GGTGCTAGCT TGCAGGCCTA GGGCATCACG ATAGTCCCCC 300

AGATCCTGGT GCCTTTCCAC GCCCCTCCCA TGTACAGTCT GCCTTTCTGA AGGAGCTGTG 360

TGGAGCTAGG AGTGTTACAG GGCTGCTGCT CGTTTGGGAG GCTGAGGCAG GACAATATGC 420

GCCTGGACAA TACGAGAACC TGCCCCCACC CTTCAAAAGT AGCTTTTCAG TATCTTACTT 480

TTCAACTTGG TCCGCCTTCT CCTGGGATGC TGGTGTCAGC AGAAGCTGAT GGGAAGTGAA 540

CCTGGGAAGT TAGCAAAGGA GGAATGGGTA GAAAGGGGAA GTGTGAGAGA AGTCCCAGAA 600

GGACTGGGGA GCTGGTGGAG AGAGGAGCCA GCCAACACAC TGCAGCCTCA TCGTCATCTC 660

CCACCTAAGG CCACCACGTC TGAGGAGCTA TGAGCCAGAG CAAGGATTTG CAGGGTAGGG 720

AAGGTGGACC GTACAGCACC GGAGACCAGC ATTGAATCAG GGAGCCAGAG GCGCTCCTGA 780

AGAATCTCAG AGGCCAGCCT TGGGCAACAC TTGGGCTGGG CACAGGGGAG GAGGAAAGGG 840

AGGGAGGGGT GGTTAACCTC TGTACCCCTC AGGATGAAAA GCTTTTGGAC TGCTGAAAGC 900

CCAGCATGGA GAGAGGTTGC TGGGATCAAC AAGCACTTCC TCGATGATTC CAAGTACAAC 960

AGTGATGAGG ATCTGCAGTC CAAACTGGAG TCCTCAAGAG TGAGGAGACT GGGGGGGGGG 1020

GGGAGCTGGA GTCTGGGGGC TCAAATCGTG GCAAGGCTGG CTGCTGTCCT GGGGAAGGAG 1080

GGAGGTGCCT TCAAGCTCCT CCTTCCCATG GTGGTCCTTT GTCCTGCTCT CCCCATCTTA 1140

GCATCCCCGC CCCCAGGCTC CCTCACAAGC TCTCTCCCCA TGCCCTCCTT CTGTATCTTC 1200

TACCCTGGCA TTGCTCAGCT GTCTCATACC GCAAACCCCA CAATGCAATT ATACTCTCTG 1260

CTCTCTTTCT ACCCAGCGAA GTACATGGAG TTTGATCTGA ATGGCAATGG AGATATCGAT 1320

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ATTATGTCCT TGAAGCGAAT GCTGGAGAAA CTTGGGGTTC CCAAGACCCA TCTAGAGCTG    1380

AAGAAATTAA TTAGAGAGTT GTCCAGTGGC TCCGAGGAGA CGTTCAGTTA CTCTGACTTT    1440

CTCAGAATGA TGCTGGGCAA GAGATCTGCC ATCTTGAGAA TGATTCTGAT GTATGAGGAG    1500

AAAAACAAGA ACACCAGAAG CCAACTGGTC CCCCAGCCAA GAAAGCTATT TCTGAGTTGC    1560

CCTAATTGGA GGTGGATATA ACACGGTGGG ACCAGGACCT TCTAATGACA GCAGCATGGG    1620

AAAAGAAGAA GCAGTTGTGA GACCAGAGTC AAACTAAATA AATAATGCTC CCTAGTGC      1678
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 696 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 143..580

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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AGACGCACCC TCTGATGTGG TCTGCACAGG GCCCTGGGCT CAGCTCACCC CATTCTTGGA      60

GCAGCCTGCA GACTTCAGAC TCTCTCTTCC CTACGGGAGA AAGTCAGCCA GTCCTCCTCA     120

GCTGCTTGTC TTAACCTGCA CC ATG AAG CCT GAG GAA ATT TCA AGA GGA AAA       172
                         Met Lys Pro Glu Glu Ile Ser Arg Gly Lys
                           1               5                  10

GCT TTT GGA CTG CTG AAA GCC CAG CAG GAA GAG AGG TTG GAT GGG ATC       220
Ala Phe Gly Leu Leu Lys Ala Gln Gln Glu Glu Arg Leu Asp Gly Ile
                 15                  20                  25

AAC AAG CAC TTC CTC GAT GAT CCC AAG TAC AGC AGT GAT GAG GAT CTG       268
Asn Lys His Phe Leu Asp Asp Pro Lys Tyr Ser Ser Asp Glu Asp Leu
             30                  35                  40

CAG TCC AAA CTG GAG GCC TTC AAG ACG AAG TAC ATG GAG TTT GAT CTG       316
Gln Ser Lys Leu Glu Ala Phe Lys Thr Lys Tyr Met Glu Phe Asp Leu
         45                  50                  55

AAT GGC AAT GGA GAT ATC GAT ATT ATG TCC TTG AAG CGA ATG CTG GAG       364
Asn Gly Asn Gly Asp Ile Asp Ile Met Ser Leu Lys Arg Met Leu Glu
     60                  65                  70

AAA CTT GGG GTT CCC AAG ACC CAT CTA GAG CTG AAG AAA TTA ATT AGA       412
Lys Leu Gly Val Pro Lys Thr His Leu Glu Leu Lys Lys Leu Ile Arg
 75                  80                  85                  90

GAG GTG TCC AGT GGC TCC GAG GAG ACG TTC AGT TAC TCT GAC TTT CTC       460
Glu Val Ser Ser Gly Ser Glu Glu Thr Phe Ser Tyr Ser Asp Phe Leu
                 95                 100                 105

AGA ATG ATG CTG GGC AAG AGA TCT GCC ATC TTG AGA ATG ATT CTG ATG       508
Arg Met Met Leu Gly Lys Arg Ser Ala Ile Leu Arg Met Ile Leu Met
             110                 115                 120
```

```
TAT GAG GAG AAA AAC AAA GAA CAC CAG AAG CCA ACT GGT CCC CCA GCC      556
Tyr Glu Glu Lys Asn Lys Glu His Gln Lys Pro Thr Gly Pro Pro Ala
        125                 130                 135

AAG AAA GCT ATT TCT GAG TTG CCC TAATTGGAGG TGGATATAAC ACGGTGGGAC    610
Lys Lys Ala Ile Ser Glu Leu Pro
    140                 145

CGAGGACCTT CTAATGACAG CAGCATGGGA AAAGAAGAAG CAGTTGTGAG CCAGAGTCAA   670

ACTAAATAAA TAATGCTCCC TGATGC                                        696
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Lys Pro Glu Glu Ile Ser Arg Gly Lys Ala Phe Gly Leu Leu Lys
 1               5                  10                  15

Ala Gln Gln Glu Glu Arg Leu Asp Gly Ile Asn Lys His Phe Leu Asp
            20                  25                  30

Asp Pro Lys Tyr Ser Ser Asp Glu Asp Leu Gln Ser Lys Leu Glu Ala
        35                  40                  45

Phe Lys Thr Lys Tyr Met Glu Phe Asp Leu Asn Gly Asn Gly Asp Ile
    50                  55                  60

Asp Ile Met Ser Leu Lys Arg Met Leu Glu Lys Leu Gly Val Pro Lys
65                  70                  75                  80

Thr His Leu Glu Leu Lys Lys Leu Ile Arg Glu Val Ser Ser Gly Ser
                85                  90                  95

Glu Glu Thr Phe Ser Tyr Ser Asp Phe Leu Arg Met Met Leu Gly Lys
            100                 105                 110

Arg Ser Ala Ile Leu Arg Met Ile Leu Met Tyr Glu Glu Lys Asn Lys
        115                 120                 125

Glu His Gln Lys Pro Thr Gly Pro Pro Ala Lys Lys Ala Ile Ser Glu
    130                 135                 140

Leu Pro
145
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 635 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 72..512

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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GGCACGAGAG CCTGCAGACA GAGGCCTCCA GCTTGGTCTG TCTCCCCACC TCTACCAGCA      60

TCTGCTGAGC T ATG AGC CAA ACC AGG GAT TTA CAG GGA GGA AAA GCT TTC      110
             Met Ser Gln Thr Arg Asp Leu Gln Gly Gly Lys Ala Phe
               1               5                  10

GGA CTG CTG AAG GCC CAG CAG GAA GAG AGG CTG GAT GAG ATC AAC AAG      158
Gly Leu Leu Lys Ala Gln Gln Glu Glu Arg Leu Asp Glu Ile Asn Lys
     15                  20                  25

CAA TTC CTA GAC GAT CCC AAA TAT AGC AGT GAT GAG GAT CTG CCC TCC      206
Gln Phe Leu Asp Asp Pro Lys Tyr Ser Ser Asp Glu Asp Leu Pro Ser
 30                  35                  40                  45

AAA CTG GAA GGC TTC AAA GAG AAA TAC ATG GAG TTT GAC CTT AAT GGA      254
Lys Leu Glu Gly Phe Lys Glu Lys Tyr Met Glu Phe Asp Leu Asn Gly
                 50                  55                  60

AAT GGC GAT ATT GAT ATC ATG TCC CTG AAA CGA ATG CTG GAG AAA CTT      302
Asn Gly Asp Ile Asp Ile Met Ser Leu Lys Arg Met Leu Glu Lys Leu
             65                  70                  75

GGA GTC CCC AAG ACT CAC CTA GAG CTA AAG AAA TTA ATT GGA GAG GTG      350
Gly Val Pro Lys Thr His Leu Glu Leu Lys Lys Leu Ile Gly Glu Val
         80                  85                  90

TCC AGT GGC TCC GGG GAG ACG TTC AGC TAC CCT GAC TTT CTC AGG ATG      398
Ser Ser Gly Ser Gly Glu Thr Phe Ser Tyr Pro Asp Phe Leu Arg Met
     95                  100                 105

ATG CTG GGC AAG AGA TCT GCC ATC CTA AAA ATG ATC CTG ATG TAT GAG      446
Met Leu Gly Lys Arg Ser Ala Ile Leu Lys Met Ile Leu Met Tyr Glu
110                 115                 120                 125

GAA AAA GCG AGA GAA AAG GAA AAG CCA ACA GGC CCC CCA GCC AAG AAA      494
Glu Lys Ala Arg Glu Lys Glu Lys Pro Thr Gly Pro Pro Ala Lys Lys
                130                 135                 140

GCT ATC TCT GAG TTG CCC TGATTTGAAG GGAAAAGGGA TGATGGGATT             542
Ala Ile Ser Glu Leu Pro
            145

GAAGGGGCTT CTAATGACCC AGATATGGAA ACAGAAGACA AAATTGTAAG CCAGAGTCAA    602

CAAATTAAAT AAATTACCCC CTCCTCCAAA AAA                                 635
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Ser Gln Thr Arg Asp Leu Gln Gly Gly Lys Ala Phe Gly Leu Leu
```

```
  1               5                  10                  15

Lys Ala Gln Gln Glu Glu Arg Leu Asp Glu Ile Asn Lys Gln Phe Leu
            20                  25                  30

Asp Asp Pro Lys Tyr Ser Ser Asp Glu Asp Leu Pro Ser Lys Leu Glu
        35                  40                  45

Gly Phe Lys Glu Lys Tyr Met Glu Phe Asp Leu Asn Gly Asn Gly Asp
    50                  55                  60

Ile Asp Ile Met Ser Leu Lys Arg Met Leu Glu Lys Leu Gly Val Pro
65                  70                  75                  80

Lys Thr His Leu Glu Leu Lys Lys Leu Ile Gly Glu Val Ser Ser Gly
                85                  90                  95

Ser Gly Glu Thr Phe Ser Tyr Pro Asp Phe Leu Arg Met Met Leu Gly
            100                 105                 110

Lys Arg Ser Ala Ile Leu Lys Met Ile Leu Met Tyr Glu Glu Lys Ala
        115                 120                 125

Arg Glu Lys Glu Lys Pro Thr Gly Pro Pro Ala Lys Lys Ala Ile Ser
    130                 135                 140

Glu Leu Pro
145
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACTTCAGACT CTCTCTTCCC TACGG 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCATCAGGGA GCATTATTTA TTTAGTTT 28

What is claimed is:

1. A method of diagnosing proliferative arterial disease characterized by excessive production of RC-9, comprising the steps of:

(a) comprising contacting a sample from a patient with a diagnostic reagent selected from the group consisting of:

(i) an antibody to RC-9;

(ii) an RC-9 polynucleotide sequence; and

(iii) an RC-9 protein;

said reagent associated with a detectable label, and

(b) detecting the presence of RC-9 in said sample by the association between said reagent and RC-9 in said sample.

2. The method according to claim 1, wherein the antibody is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a Fab fragment, and a single chain antibody.

3. The method according to claim 2, wherein the antibody is a monoclonal antibody.

4. The method according to claim 1 wherein the polynucleotide sequence is selected from the group consisting of:

(a) a DNA sequence comprising SEQ ID NO:1;

(b) a DNA sequence comprising SEQ ID NO: 2;

(c) a DNA sequence comprising SEQ ID NO: 3;

(d) a DNA sequence comprising SEQ ID NO: 4;

(e) an RNA sequence corresponding to the DNA sequence of (a) - (d) above;

(f) sequences capable of hybridizing to (a) - (d) under stringent conditions; and

(g) fragments and analogs of (a) - (d) having a RC-9-like biological activity.

5. The method according to claim 1, wherein the RC-9 protein is selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4;

(b) the amino acid sequence of SEQ ID NO:6;

(c) analogs of (a) or (b); and

(d) a fragment of (a) or (b) comprising 5 amino acids and characterized by RC-9-like biological activity.

6. A method of treating proliferative arterial disease characterized by excessive production of RC-9, comprising the step of administering to a patient a pharmaceutical composition comprising an antibody to RC-9.

7. The method according to claim 6, wherein the antibody is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a Fab fragment, and a single chain antibody.

8. The method according to claim 7, wherein the antibody is a monoclonal antibody.

9. A method of identifying compounds which specifically bind to a selected RC-9 DNA sequence, comprising the steps of contacting said RC-9 DNA sequence with a test compound to permit binding of the test compound to the DNA sequence; and determining the amount of test compound which is bound to the DNA sequence.

10. The method according to claim 10 wherein said RC-9 DNA sequence is immobilized on a solid support.

11. A method of identifying compounds which specifically bind to a selected RC-9 amino acid sequence, comprising the steps of contacting said RC-9 amino acid sequence with a test compound to permit binding of the test compound to the amino acid sequence; and determining the amount of test compound which is bound to the amino acid sequence.

12. The method according to claim 10 wherein said RC-9 amino acid sequence is immobilized on a solid support.

13. A method of identifying compounds which specifically bind to a selected RC-9 antibody, comprising the steps of contacting said RC-9 antibody with a test compound to permit binding of the test compound to the antibody; and determining the amount of test compound which is bound to the antibody.

14. The method according to claim 10 wherein said RC-9 antibody is immobilized on a solid support.

15. A compound capable of inhibiting the activity of RC-9, produced by screening a group of compounds with a RC-9 protein, RC-9 DNA sequence or anti-RC-9 antibody.

16. Use of an RC-9 antibody in the preparation of a medicament for the treatment of proliferative arterial disease characterized by excessive production of RC-9.

FIGURE 1

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AGCCAGCGAA GTACATGGAG TTTGATCTGA ATGGCAATGG AGATATCGAT ATTATGTCCT      60
TGAAGCGAAT GCTGGAGAAA CTTGGGGTTC CCAAGACCCA TCTAGAGCTG AAGAAATTAA     120
TTAGAGAGTT GTCCAGTGGC TCCGAGGAGA CGTTCAGTTA CTCTGACTTT CTCAGAATGA     180
TGCTGGGCAA GAGATCTGCC ATCTTGAGAA TGATTCTGAT GTATGAGGAG AAAAACAAAG     240
AACACCAGAA GCCAACTGGT CCCCCAGCCA AGAAAGCTAT TTCTGAGTTG GCCTAATGGA     300
GGTGGATATA ACACGGTGGG ACCGAGGACC TTCGTGTCAG CAGCATGGGA AGAGAAGAAT     360
CAGTTGCGAG CCAGACTGTC AATGCTCAAT AAATAATGCT CCCTAGTGCC TGAAAAAAAA     420
AAAA                                                                 424
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FIGURE 2

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GTCTCACCCC ATTCTTGGAG CAGCCTGCAG ACTTCAGACT CTCTCTTCCC TACGGAGAAA      60

GTCAGCCAGT CCTCCTCAGC TGCTTCTTAA CCTGCACCAT GAAGCCTGAG GAAATTTCAA     120

                    INTRON 1
GTAACCCTCC CAGCCCCCAC CCCAGGGTCT GGGGAAGAAG TCATTTTTGT GTTTCCTTGG     180

TTTGGGTTTG AGACAGGTTC TTACTGTGTA GGCCTGGCTG GTCTCCAACT CCCAGGATCC     240

TCTAGCCTCA ACCTCTCTTA GGTGCTAGCT TGCAGGCCTA GGGCATCACG ATAGTCCCCC     300

AGATCCTGGT GCCTTTCCAC GCCCCTCCCA TGTACAGTCT GCCTTTCTGA AGGAGCTGTG     360

TGGAGCTAGG AGTGTTACAG GGCTGCTGCT CGTTTGGGAG GCTGAGGCAG GACAATATGC     420

GCCTGGACAA TACGAGAACC TGCCCCCACC CTTCAAAAGT AGCTTTTCAG TATCTTACTT     480

TTCAACTTGG TCCGCCTTCT CCTGGGATGC TGGTGTCAGC AGAAGCTGAT GGGAAGTGAA     540

CCTGGGAAGT TAGCAAAGGA GGAATGGGTA GAAAGGGGAA GTGTGAGAGA AGTCCCAGAA     600

GGACTGGGGA GCTGGTGGAG AGAGGAGCCA GCCAACACAC TGCAGCCTCA TCGTCATCTC     660

CCACCTAAGG CCACCACGTC TGAGGAGCTA TGAGCCAGAG CAAGGATTTG CAGGGTAGGG     720

AAGGTGGACC GTACAGCACC GGAGACCAGC ATTGAATCAG GGAGCCAGAG GCGCTCCTGA     780

AGAATCTCAG AGGCCAGCCT TGGGCAACAC TTGGGCTGGG CACAGGGGAG GAGGAAAGGG     840

      Branch Point          Poly C
   Consensus Sequence       Tract
AGGGAGGGGT GGTTAACCTC TGTACCCCTC AGGATGAAAA GCTTTTGGAC TGCTGAAAGC     900

CCAGCATGGA GAGAGGTTGC TGGGATCAAC AAGCACTTCC TCGATGATTC CAAGTACAAC     960

                                                  INTRON 2
AGTGATGAGG ATCTGCAGTC CAAACTGGAG TCCTCAAGAG TGAGGAGACT GGGGGGGGGG    1020

GGGAGCTGGA GTCTGGGGGC TCAAATCGTG GCAAGGCTGG CTGCTGTCCT GGGGAAGGAG    1080

GGAGGTGCCT TCAAGCTCCT CCTTCCCATG GTGGTCCTTT GTCCTGCTCT CCCCATCTTA    1140

GCATCCCCGC CCCCAGGCTC CCTCACAAGC TCTCTCCCCA TGCCCTCCTT CTGTATCTTC    1200

      Branch Point
  Consensus _______ Sequence                                  Poly
TACCCTGGCA TTGCTCAGCT GTCTCATACC GCAAACCCCA CAATGCAATT ATACTCTCTG    1260

 C Tract
CTCTCTTTCT ACCCAGCGAA GTACATGGAG TTTGATCTGA ATGGCAATGG AGATATCGAT    1320

ATTATGTCCT TGAAGCGAAT GCTGGAGAAA CTTGGGGTTC CCAAGACCCA TCTAGAGCTG    1380

AAGAAATTAA TTAGAGAGTT GTCCAGTGGC TCCGAGGAGA CGTTCAGTTA CTCTGACTTT    1440

CTCAGAATGA TGCTGGGCAA GAGATCTGCC ATCTTGAGAA TGATTCTGAT GTATGAGGAG    1500

AAAAACAAGA ACACCAGAAG CCAACTGGTC CCCCAGCCAA GAAAGCTATT TCTGAGTTGC    1560

CCTAATTGGA GGTGGATATA ACACGGTGGG ACCAGGACCT TCTAATGACA GCAGCATGGG    1620

AAAAGAAGAA GCAGTTGTGA GACCAGAGTC AAACTAAATA AATAATGCTC CCTAGTGC      1678
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FIGURE 3

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AGACGCACCC TCTGATGTGG TCTGCACAGG GCCCTGGGCT CAGCTCACCC CATTCTTGGA      60

GCAGCCTGCA GACTTCAGAC TCTCTCTTCC CTACGGGAGA AAGTCAGCCA GTCCTCCTCA     120

GCTGCTTGTC TTAACCTGCA CC ATG AAG CCT GAG GAA ATT TCA AGA GGA AAA       172
                         Met Lys Pro Glu Glu Ile Ser Arg Gly Lys
                           1               5                  10

GCT TTT GGA CTG CTG AAA GCC CAG CAG GAA GAG AGG TTG GAT GGG ATC       220
Ala Phe Gly Leu Leu Lys Ala Gln Gln Glu Glu Arg Leu Asp Gly Ile
                  15                  20                  25

AAC AAG CAC TTC CTC GAT GAT CCC AAG TAC AGC AGT GAT GAG GAT CTG       268
Asn Lys His Phe Leu Asp Asp Pro Lys Tyr Ser Ser Asp Glu Asp Leu
              30                  35                  40

CAG TCC AAA CTG GAG GCC TTC AAG ACG AAG TAC ATG GAG TTT GAT CTG       316
Gln Ser Lys Leu Glu Ala Phe Lys Thr Lys Tyr Met Glu Phe Asp Leu
          45                  50                  55

AAT GGC AAT GGA GAT ATC GAT ATT ATG TCC TTG AAG CGA ATG CTG GAG       364
Asn Gly Asn Gly Asp Ile Asp Ile Met Ser Leu Lys Arg Met Leu Glu
      60                  65                  70

AAA CTT GGG GTT CCC AAG ACC CAT CTA GAG CTG AAG AAA TTA ATT AGA       412
Lys Leu Gly Val Pro Lys Thr His Leu Glu Leu Lys Lys Leu Ile Arg
  75                  80                  85                  90

GAG GTG TCC AGT GGC TCC GAG GAG ACG TTC AGT TAC TCT GAC TTT CTC       460
Glu Val Ser Ser Gly Ser Glu Glu Thr Phe Ser Tyr Ser Asp Phe Leu
                  95                 100                 105

AGA ATG ATG CTG GGC AAG AGA TCT GCC ATC TTG AGA ATG ATT CTG ATG       508
Arg Met Met Leu Gly Lys Arg Ser Ala Ile Leu Arg Met Ile Leu Met
              110                 115                 120

TAT GAG GAG AAA AAC AAA GAA CAC CAG AAG CCA ACT GGT CCC CCA GCC       556
Tyr Glu Glu Lys Asn Lys Glu His Gln Lys Pro Thr Gly Pro Pro Ala
          125                 130                 135

AAG AAA GCT ATT TCT GAG TTG CCC TAA TTGGAGGTGG ATATAACACG            603
Lys Lys Ala Ile Ser Glu Leu Pro
      140                 145

GTGGGACCGA GGACCTTCTA ATGACAGCAG CATGGGAAAA GAAGAAGCAG TTGTGAGCCA     663

GAGTCAAACT AAATAAATAA TGCTCCCTGA TGC                                  696
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FIGURE 4

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GGCACGAGAG CCTGCAGACA GAGGCCTCCA GCTTGGTCTG TCTCCCCACC                    50

TCTACCAGCA TCTGCTGAGC T ATG AGC CAA ACC AGG GAT TTA CAG                    95
                        Met Ser Gln Thr Arg Asp Leu Gln
                          1               5

GGA GGA AAA GCT TTC GGA CTG CTG AAG GCC CAG CAG GAA GAG                   137
Gly Gly Lys Ala Phe Gly Leu Leu Lys Ala Gln Gln Glu Glu
     10                  15                  20

AGG CTG GAT GAG ATC AAC AAG CAA TTC CTA GAC GAT CCC AAA                   179
Arg Leu Asp Glu Ile Asn Lys Gln Phe Leu Asp Asp Pro Lys
         25                  30                  35

TAT AGC AGT GAT GAG GAT CTG CCC TCC AAA CTG GAA GGC TTC                   221
Tyr Ser Ser Asp Glu Asp Leu Pro Ser Lys Leu Glu Gly Phe
             40                  45                  50

AAA GAG AAA TAC ATG GAG TTT GAC CTT AAT GGA AAT GGC GAT                   263
Lys Glu Lys Tyr Met Glu Phe Asp Leu Asn Gly Asn Gly Asp
                 55                  60

ATT GAT ATC ATG TCC CTG AAA CGA ATG CTG GAG AAA CTT GGA                   305
Ile Asp Ile Met Ser Leu Lys Arg Met Leu Glu Lys Leu Gly
 65                  70                  75

GTC CCC AAG ACT CAC CTA GAG CTA AAG AAA TTA ATT GGA GAG                   347
Val Pro Lys Thr His Leu Glu Leu Lys Lys Leu Ile Gly Glu
     80                  85                  90

GTG TCC AGT GGC TCC GGG GAG ACG TTC AGC TAC CCT GAC TTT                   389
Val Ser Ser Gly Ser Gly Glu Thr Phe Ser Tyr Pro Asp Phe
         95                  100                 105

CTC AGG ATG ATG CTG GGC AAG AGA TCT GCC ATC CTA AAA ATG                   431
Leu Arg Met Met Leu Gly Lys Arg Ser Ala Ile Leu Lys Met
             110                 115                 120

ATC CTG ATG TAT GAG GAA AAA GCG AGA GAA AAG GAA AAG CCA                   473
Ile Leu Met Tyr Glu Glu Lys Ala Arg Glu Lys Glu Lys Pro
                 125                 130

ACA GGC CCC CCA GCC AAG AAA GCT ATC TCT GAG TTG CCC                       512
Thr Gly Pro Pro Ala Lys Lys Ala Ile Ser Glu Leu Pro
135                 140

TGATTTGAAG GGAAAAGGGA TGATGGGATT GAAGGGGCTT CTAATGACCC                    562

AGATATGGAA ACAGAAGACA AAATTGTAAG CCAGAGTCAA CAAATTAAAT                    612

AAATTACCCC CTCCTCCAAA AAA                                                 635
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19671

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : GO1N 33/563, 33/559, 33/537, 33/531, 33/53, 33/542; C12Q 1/68
US CL : 436/512, 515, 538, 543, 547; 435/6, 7.1, 7.9
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/512, 515, 538, 543, 547; 435/6, 7.1, 7.9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, EMBASE, CA, BIOSIS
search terms proliferative arterial disease, RC9 protein

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AUTIERI et al. Use of Differential Display to identify differentially expressed mRNAs induced by rat carotid artery balloon angioplasty. Laboratory Investigation. June 1985, Vol 72. No. 6, pages 656-661, whole article.	1-5
Y	UTANS et al. Cloning and characterization of Allograft Inflammatory Factor-1: A novel macrophage factor identified in rat cardiac allografts with chronic rejection. J. Clin. Inves. June 1995, Vol. 95, No.6, pages 2954-2962, whole article.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	
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"&" document member of the same patent family	

Date of the actual completion of the international search 25 FEBRUARY 1997	Date of mailing of the international search report 21 MAR 1997
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Form PCT/ISA/210 (second sheet)(July 1992)*