Assessment of Endostatin Gene Therapy for Familial Adenomatous Polyposis–Related Desmoid Tumors

Sandra C.M. Martinico,1 Sarah Jezzard,1 N. Julian H. Sturt,2 Genevieve Michils,3 Sabine Tejpar,3 Robin K. Phillips,3 and Georges Vassaux1

1Institute of Cancer and Cancer Research UK Clinical Centre, Barts and The London Queen Mary's School of Medicine and Dentistry; 2Polyposis Registry, Cancer Research UK Colorectal Cancer Unit, St. Mark's Hospital, London, United Kingdom; and 3Center for Human Genetics, Laboratorium voor Moleculaire Diagnostiek, Leuven, Belgium

Abstract
Constitutive activation of the Wnt signaling pathway is a hallmark of many cancers, including familial adenomatous polyposis (FAP)–related desmoid tumors. Endostatin is a well-known antiangiogenic protein that has been described recently as a potential inhibitor of this signaling pathway. Here, we show that endostatin directly induces apoptosis and inhibits the Wnt signaling pathway in colorectal cancer cell lines bearing mutations on the adenomatous polyposis coli (APC) gene as a model of FAP-related malignant cells. We then explore the relationship between apoptosis and inhibition of this pathway and show that they are not correlated. These results seem to contradict a well-recognized study, showing that reintroduction of the APC cDNA in APC-deficient cells leads to apoptosis. To reconcile our conclusions with the literature, we further show that a truncated fragment of APC capable of inhibiting the Wnt signaling pathway in SW480 cells is incapable of inducing apoptosis in these cells, confirming that APC-mediated apoptosis is uncoupled to the inhibition of the Wnt signaling pathway. Finally, we show that endostatin directly induces cell death on primary FAP-related desmoid tumor cells in culture. This phenomenon is also independent of the inhibition of the Wnt signaling pathway. Considering the current lack of effective treatment against desmoid tumors, we advocate that endostatin gene therapy represents an attractive new therapeutic approach for this disease. (Cancer Res 2006; 66(16): 8233–40)

Introduction
Desmoid tumors are locally aggressive monoclonal proliferations of fibroblast-like cells (1, 2). Their cortex is highly vascularized and surgical resection is suspected to induce recurrence of the tumor (3). Familial adenomatous polyposis (FAP) patients present an increased risk of developing these tumors than the rest of the population (4, 5). These patients generally develop several hundred polyps in the gastrointestinal tract and desmoids often grow intra-abdominally, leading to major morbidity and mortality (6, 7). This inherited disease is linked, like most sporadic colorectal cancers (~80%), to nonsense or frameshift mutations of the tumor suppressor gene adenomatous polyposis coli (APC; refs. 8–10). The direct consequence is the expression of a truncated inactive form of the protein. APC is a key component of the Wnt signaling pathway, which is notably involved in the intestine physiology. A tight regulation of the pathway is necessary for the formation of normal crypt-villus morphology (11–13). The activated pathway induces accumulation of β-catenin in the cytoplasm and nucleus of cells, where it binds to members of the Tcf/Lef family of transcription factors and activates transcription of genes involved in cell proliferation, such as c-myc and cyclin D1 (14, 15). APC forms a complex with proteins, such as axin and glycogen synthase 3β, to trigger degradation of β-catenin through the ubiquitin-proteasome pathway and therefore acts as an inhibitor of the Wnt signaling pathway (16–18).

There is currently no satisfactory treatment for FAP-related desmoids. Surgery is very challenging and therefore usually done when the tumor size becomes life threatening. There is a need for alternative or complimentary therapies that could delay surgery if not eradicate the tumor. Intradatum injection of a gene therapy formulation could be a beneficial therapeutic approach for such a genetic disorder. An interesting approach would combine inhibition of the Wnt signaling pathway with an antiangiogenic action and endostatin may be an attractive transgene.

Endostatin, a 20-kDa protein, derives from the proteolysis of collagen XVIII COOH-terminal domain. It has antiangiogenic activity that inhibits tumor growth in vivo (19). In addition, endostatin has been shown to exert a direct antitumor effect on colon cancer cell lines and preestablished tumors in murine models (20). Moreover, phase I clinical trials have been completed to assess the safety and pharmacokinetics of endostatin in patients with solid tumors. Both studies concluded that endostatin is well tolerated with no significant drug-related toxicity observed (21, 22). Although its mechanism of action remains unclear, it has been suggested to act as an inhibitor of the Wnt signaling pathway (23, 24) triggering β-catenin degradation, which makes endostatin even more attractive as candidate for gene therapy against FAP-associated desmoids. However, endostatin has a short half time (25) and a successful therapy would require prolonged expression of endostatin in vivo. Among them, the use of viral vectors, such as adenoviruses, has proven to be efficient. This technology enables high expression of endostatin for several days (27).

The present study aims at comparing the effects of endostatin with other known inhibitors of the Wnt signaling pathway (APC and axin) on established colorectal cancer cell lines to show the infectability of primary cells obtained from FAP-related desmoid tumors by recombinant adenovirus and to assess the effect of adenovirus-mediated endostatin expression on these primary cells.
Materials and Methods

**Cell culture.** 293 cells and colon cancer cell lines SW480 and DLD1 were obtained from Cancer Research UK Research Cell Services department (London, United Kingdom). HCT116 p53+/− and p53−/− were kindly provided by Prof. Vogelstein (Department of Pathology, Johns Hopkins Medical Institution, Baltimore, MD). Two hundred sixty-three CymR cells were purchased from Qbiogene (San Diego, CA). Cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; AutogenBioclear, Ltd., Calne, United Kingdom), 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.2 μg/mL butyl-p-hydroxybenzoate. The POT cell line generated from DLD1 was maintained in DMEM supplemented with 10% FBS and 0.5 mg/mL gentamicin (Invitrogen, Paisley, United Kingdom).

**Establishment of stable cell lines.** POT cell line was derived from DLD1 cells by cotransfection of pcDNA3 plasmid from Invitrogen, and a Tcf-4-responsive luciferase plasmid pGL3-OT was generously provided by Prof. Vogelstein (Department of Pathology, Johns Hopkins Medical Institution, Baltimore, MD). Two hundred sixty-three CymR cells (p53−/−) were infected with AdLacZ, AdEndo, AdAxin, or AdtAPC at 100 MOI. NI, uninfected control. Cells were harvested 48 hours after infection, and Western blot was done for detection of axin, tAPC (anti-V5 tag), β-catenin, Ku-70 (loading control), and c-myc (SW480 only; see Materials and Methods for details of primary antibodies). These blots are examples of typical observations representative of at least three experiments.

**Establishment of primary cultures of desmoid cells.** Desmoid tumor samples were obtained from St Mark's Hospital (London, United Kingdom) following surgical resection. The protocol was used in conjunction with ethical approval from the Harrow Research Ethics Committee. The tumors were macerated in small pieces with a scalpel and digested overnight at 37°C in RPMI 1640 containing 20% FBS and antibiotics (see above) supplemented with 1 mg/mL collagenase D (Roche Diagnostics GmbH, Lewes, United Kingdom). The digest was then filtered through a 100-μm nylon cell strainer and centrifuged twice at 1,000 rpm. Cell pellets were washed twice with PBS, resuspended in RPMI 1640 and 20% FBS, and seeded. Primary cultures were maintained in a 5% CO2 atmosphere at 37°C. Erythrocytes and cell debris were washed away with PBS 24 hours later. Germ line and somatic mutations were identified: Desmoid 3: germ line exon 15G c.3927-3931del AAAGA (codon 1309), somatic exon 15H: c.4382 G > T (p.G1428X); Desmoid 5: germ line exon 6 c.646 C > T (p.R216X), somatic exon 15H: c.4385delA.

**DNA oligonucleotides.** Small interfering RNA (siRNA) oligonucleotides directed against β-catenin were obtained from Dharmacon (Lafayette, CO). Three siRNAs against β-catenin were tested and the most potent at reducing β-catenin protein levels was selected (data not shown). 5′-GAGGGAAGGAGGAUUGdTDdT-3′; 5′-CACACUCUCUCCUCUCAGGdTDdT-3′. 5′-CCUCUGGAAGGAGGAUUGdTDdT-3′ extended between nucleotides 365 and 385 of β-catenin mRNA. siRNAs directed against the green fluorescent protein (GFP) or the firefly luciferase (Dharmacon) were used as controls.

**Transfection of siRNAs.** Cells were seeded in culture medium without antibiotics at a cell density of 104 per cm2. The following day, the cells were transfected for 4 hours with Oligofectamine reagent (Invitrogen) combined with siRNAs at a concentration of 300 nmoL/L in reduced serum medium Opti-MEM I (Invitrogen). Cells were then fed with culture medium without antibiotic to a final concentration of 10% FBS (cell lines) or 20% (desmoid cells). Cells were assayed at 2, 3, or 5 days after transfection.

**Generation of recombinant adenoviruses encoding TAP (AdtAPC) and axin (AdAxin).** A plasmid containing the full-length APC (pAPC) was kindly provided by Prof. Vogelstein. The plasmid containing the rat axin (pEF-BOS-rAxin) was a gift from Dr. Kypta (Imperial College London, United Kingdom). Fragments of APC (amino acid 1034-1698) and axin (full length) were isolated by PCR. The primer oligonucleotide sequences were as follows: APC, 5′-ACCATGGAGCAGTTGAACTCTGGAGG-3′ (upstream) and 5′-ATCTGTACTTCTGCCTTCTGTAGG-3′ (downstream) and axin, 5′-GCGGCCGCACTGACAGTCCCAAAATGAATG-3′ (upstream) and 5′-TCAATCCTCCTCGTCCGTTGAGG-3′ (downstream). An NcoI (for APC) or NotI site (for axin) was introduced at the 5′-end of the upstream primer to allow afterward subcloning. The PCR products were cloned in the pcDNA3.1/V5-His plasmid from the Invitrogen pcDNA3.1/V5-His TOPO TA Expression kit (see the manufacturer’s instructions), the resulting

![Figure 1. Expression of transgenes and effect on β-catenin and c-myc protein levels](image1)

![Figure 2. Effect of adenoviruses encoding Wnt signaling inhibitors on the transcriptional activity of β-catenin/Tcf complex. Stable DLD1-POT cells](image2)
plasmids are ptAPC and pAxin. The coding sequences were then sub-cloned into the AdEasy shuttle system [double sequential digestion BamHI/PmeI for ptAPC and NotI/EcoRV for pAxin was cloned, respectively, into pAdenovator-CMV (Qbiogene) linearized by BglII/EcoRV and pShuttle-CMV linearized by NotI/EcoRV]. The resulting plasmids were used to generate recombinant replicative-deficient adenoviruses by homologous recombination with pAdEasy-1 in Escherichia coli BJ5183 (Qbiogene). The recombinant viruses (AdtAPC and AdAxin) were produced in 293CymR or 293 adenovirus packaging cells, respectively. Replication-incompetent adenovirus encoding the murine endostatin (AdEndo) was purchased from Qbiogene. The viral particles were purified, and titers were determined as described previously (29).

**Infections with adenoviruses.** Cells from established lines were seeded at a cell density of 3 x 10^4 per cm² and at 10^5 per cm² for primary cultures. The following day, they were infected with recombinant replication-incompetent adenoviruses at different multiplicities of infection (MOI) in serum-free medium for 30 to 45 minutes. Culture medium was then added to reach a serum concentration up to 10% (cell lines) or 20% (desmoid primary cells).

**Biochemical assays.** Western blot analysis and assessment of apoptosis were done according to Stoll et al. (30). Primary antibodies used in this study are the following: anti-β-catenin (E-5) monoclonal antibody and anti-Ku-70 (C19) and anti-axin (H-98) polyclonal antibodies from Santa Cruz Biotechnology (Calne, United Kingdom). Anti-V5 monoclonal antibody was purchased from Invitrogen, and anti-c-myc clone 9E10 was obtained from Cancer Research UK monoclonal antibody service. Endostatin concentration in the tissues culture medium was measured using the endostatin ELISA test (AMS Biotechnology, Oxon, United Kingdom). Cell survival and β-galactosidase assays were done as described previously (31). To characterize the signaling pathway involved in endostatin-mediated apoptosis, the Oligo GEArray “Human Signal Transduction PathwayFinder Microarray” was used (GEArray, Frederick, MD). The experiments were done as described in the manufacturer’s protocol.

**Results**

**Characterization of adenoviruses encoding endostatin, axin, or tAPC.** Western blot analysis of proteins extracted from

---

**Figure 3.** Wnt signaling inhibitors activity on cell survival of APC-deficient colorectal cancer cells. **A,** SW480 and DLD1 cells were infected at 50 and 100 MOI with AdLacZ, AdEndo, AdAxin, or AdtAPC. Cell viability was assessed 48 hours after infection by measuring the cell metabolism with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. Results are percentage relative viability to infection control (100% viability with AdLacZ), *, P < 0.05; **, P < 0.005; ***, P < 0.0005, determined with a two-tailed Student’s t test. Representative of at least two independent experiments done in triplicates. **B** and **C,** SW480 cells were infected at 50 MOI with AdLacZ, AdEndo, AdAxin, or AdtAPC, NI, uninfected control. Apoptosis was assessed by flow cytometry with two endpoints: plasma membrane destabilization by Annexin V staining 48 hours after infection (B) and DNA fragmentation by sub-G1 analysis 72 hours after treatment (C). This experiment has been repeated once with similar results.
the APC-deficient colorectal cell line SW480 infected with the adenoviruses AdAxin or AdtAPC showed that axin and tAPC transgenes were efficiently expressed (Fig. 1A). In addition, high levels of endostatin were detected by ELISA in the supernatant of cells infected with AdEndo (data not shown). As expected (23, 28, 32), expression of tAPC, axin, and endostatin resulted in reduced levels of β-catenin protein levels that was accompanied by a reduction in c-myc expression (a known downstream target of β-catenin/Tcf-4 complex; Fig. 1A).

Transgene expression was also detected on infection of the APC-deficient DLD1 colorectal cell line with AdAxin or AdtAPC (Fig. 1B) and high levels of endostatin were detected by ELISA in the supernatant of cells infected with AdEndo (data not shown). Expression of axin and endostatin induced a reduction of β-catenin protein levels (Fig. 1B), but surprisingly, β-catenin levels in DLD1 infected with AdtAPC remained identical to those obtained on infection with AdLacZ (Fig. 1B). These results suggest that tAPC is not an inhibitor of the Wnt signaling pathway in this cell line. To confirm this hypothesis, a stable DLD1 cell line, containing a plasmid encoding the firefly luciferase downstream of Tcf-responsive elements, was established (DLD1-POT). Measurement of luciferase activity showed that endostatin and axin were capable of inhibiting the transcriptional activity of the β-catenin/Tcf complex, whereas tAPC did not (Fig. 2). Altogether, these data suggest that endostatin and axin are inhibitors of the Wnt signaling pathway, whereas tAPC inhibitory activity is cell line dependent.

**Endostatin induces apoptosis of APC-deficient colorectal cancer cells.** Infection of SW480 and DLD1 cells with AdEndo or AdAxin induced cell death in a dose-dependent manner (35-50% at 50 MOI and 50-65% at 100 MOI; Fig. 3A). This cell death was characterized by flow cytometry on SW480 cells. Annexin V staining was quantitated 48 hours after infection and showed increased plasma membrane destabilization in AdEndo- or AdAxin-infected cells, whereas AdLacZ-infected cells showed limited Annexin V staining (Fig. 3B). Very similar data were obtained when sub-G1 analysis of DNA fragmentation 72 hours after infection was done (Fig. 3C). Similar results were obtained on infection of DLD1 cells with AdAxin and AdEndo (data not shown). These data suggest expression of endostatin or axin induces apoptosis. As both cell lines used are p53 deficient, we conclude this apoptosis is p53 independent.

![Figure 4](image-url)

**Figure 4.** Effect of adenoviruses encoding Wnt signaling inhibitors on APC WT β-catenin-mutated colorectal cancer cells. HCT116 cells were infected at 100 (A) or 50 (B and C) MOI with AdLacZ, AdEndo, AdAxin, or AdtAPC. NI, uninfected control. A, forty-eight hours after treatment, cells were harvested, and Western blot for the detection of β-catenin and Ku-70 (loading control) was done. B and C, apoptosis was assessed by flow cytometry with two end points: plasma membrane destabilization by Annexin V staining 48 hours after infection (B) and DNA fragmentation by sub-G1 analysis 72 hours after treatment (C). This experiment has been repeated once with similar results.
AdEndo-mediated apoptosis is independent from inhibition of Wnt signaling pathway. In contrast to infection with AdEndo or AdAxin, infection of SW480 and DLD1 with AdtAPC did not lead to reduced cell survival (Fig. 3A), increased Annexin V staining (Fig. 3B), or increased DNA fragmentation (Fig. 3C). AdtAPC acts as an inhibitor of the Wnt signaling pathway on SW480, these data partly suggest that AdEndo-mediated apoptosis is independent from the inhibition of the Wnt signaling pathway.

To test this hypothesis, the effect of AdtAPC, AdEndo or AdAxin was assessed on the colorectal cancer cell line HCT116 [wild-type (WT) APC and β-catenin mutated]. Expression of the transgenes did not reduce the β-catenin levels (Fig. 4A), however, AdEndo and AdAxin increased plasma membrane destabilization (Fig. 4B) and DNA fragmentation (Fig. 4C), whereas infection with AdtAPC did not affect these variables (Fig. 4B and C). HCT116 cells appeared more sensitive to AdEndo and AdAxin infection than SW480 or DLD1 cells but this difference was not due to p53 expression in HCT116, as p53-negative HCT116 cells showed similar sensitivity to AdAxin and AdEndo infection than normal HCT116 (data not shown).

To further investigate the relationship between apoptosis and Wnt signaling pathway, SW480 cells were transfected with a siRNA against β-catenin (Fig. 5). Transfection of this siRNA led to a dramatic decrease in β-catenin protein levels measured by Western blot, resulting in a total absence of β-catenin 48 hours after transfection (Fig. 5A). In these conditions, SW480 cells lacking β-catenin did not show any significant increase in membrane permeability (Fig. 5B) or DNA fragmentation (Fig. 5C), suggesting a lack of apoptosis induced by the β-catenin siRNA. Similar data were obtained on DLD1 and HCT116 cells (data not shown). By contrast and consistently with a previous study (33), transfection of this siRNA resulted in a cell cycle arrest in G1 on these three cell lines (Fig. 5D; data not shown). Taken together, these results clearly show that AdEndo triggers apoptosis on colorectal cancer cells independently from inhibiting the Wnt signaling pathway.

Mechanistical aspect of endostatin-induced apoptosis. Survivin, a member of the inhibitor of apoptosis protein family, is overexpressed in many cancers and has been proposed as a target for anticancer treatments (34, 35). Down-regulation of
survivin leads to an increased apoptosis rate and to a decreased tumor growth (35–38). Moreover, survivin is an important protein for the intestine physiology and seems to be at least partially regulated through the Wnt signaling pathway in the colon (39). In addition, a potent chemotherapeutic drug used for colorectal cancer treatment (oxaliplatin) has been shown to inhibit survivin (40). We therefore assessed whether endostatin induced a reduction of survivin protein levels in colorectal cancer cell lines. Western blot analysis of cell extracts from DLD1 cells infected with AdAxin or AdEndo revealed a slight but significant reduction of surviving protein levels (data not shown). However, as transfection of siRNA against β-catenin produced a similar effect on DLD1 cells (data not shown), it is very likely that this slight reduction in survivin levels is mediated by inhibition of the Wnt signaling pathway and is unlikely to be a determinant effecter in endostatin-mediated apoptosis.

To characterize the signaling pathway involved in endostatin-mediated apoptosis, RNAs collected from SW480 cells infected with AdLacZ (control) or AdEndo were labeled and used as probe on the Oligo GEArray Human Signal Transduction PathwayFinder Microarray. The results failed to highlight a particular signaling pathway activated by endostatin (data not shown).

**Endostatin as potential gene therapy for FAP-related desmoid tumors.** Primary cultures of FAP-related desmoid tumors were established from samples obtained from two male patients (19 and 37 years old). Germ line and somatic mutations of APC have been identified (see Materials and Methods). Immunohistochemistry on tissues blocks and immunocytochemistry on primary cultures showed overexpression of β-catenin in the nucleus and cytoplasm of cells (data not shown).

To test the efficacy of adenoviral-mediated gene transfer, desmoid cells were infected with various doses of AdLacZ. Forty-eight hours later, the cells were stained for β-galactosidase expression. We observed a dose-dependent expression of the reporter gene, with no transduced cells at a MOI below 10, only ~40% to 50% of cells transduced at a MOI of 100 and ~80% of the cell population expressing the reporter gene at 500 MOI (data not shown). Interestingly, this gene expression was not accompanied by noticeable toxicity at 1,000 MOI.

On these primary desmoid cells, adenovirus-mediated endostatin delivery did not result in a significant reduction in β-catenin protein levels as opposed to transfection of siRNA against β-catenin (Fig. 6A). However, infection with AdEndo resulted in a dramatic the induction of cell death (Fig. 6B). As expected from our study on established cell lines (Fig. 5) and previous work on desmoid tumor cells transfected with the APC cDNA (41), transfection of these primary desmoid cells with siRNA against β-catenin did not result in cell death (Fig. 6C).

**Discussion**

The Wnt signaling pathway is generally thought to be constitutively active in colorectal cancer cells and this constitutive activity is also thought to be the main driver of FAP-related pathologies. Attempting to inhibit this pathway can therefore be expected to provide some therapeutic benefits in patients affected by these pathologies. However, analysis of the literature in the field provides some conflicting information on the effects of the inhibition of the Wnt signaling pathway at the cellular level. On the one hand, induction of the expression of the full-length APC cDNA in APC-deficient cells has been shown to induce apoptosis (42), an effect assumed to be mediated by the inhibition of the Wnt signaling pathway (42). On the other hand, experiments using expression of dominant-negative Tcf-4 showed that colorectal cancer cells were blocked in the cell cycle in response to the disruption of the Wnt signaling pathway (43). In addition, delivery of siRNA against β-catenin failed to activate apoptosis in these cells (33). In this context, our results support the view that the inhibition of the Wnt signaling pathway does not lead primarily to...
apoptosis (Figs. 1, 3, and 5) but arrests the cells in G1 of the cells cycle (Fig. 5D). Consistently with these conclusions, we show that a fragment of the APC gene corresponding to amino acids 1034 to 1698 of the APC protein and capable of inhibiting the Wnt signaling pathway also failed to induce apoptosis in a cell line containing endogenous inactive APC alleles (Figs. 1 and 3). Altogether, these observations (Figs. 3 and 5; refs. 33, 42, 43) suggest that inhibition of the Wnt signaling pathway is not sufficient to induce apoptosis and that a part of the APC protein located outside amino acids 1034 to 1698 is involved in APC-mediated apoptosis. Fragments of the APC protein outside this region are involved in cell migration and adhesion, in cytoskeleton regulation, and in chromosome regulation and segregation (44). It is therefore likely that the reintroduction of one or more of these APC functions into APC-deficient cancer cells may lead to apoptosis. The precise location of the APC fragment capable of inducing apoptosis is currently being investigated.

Although adrenoviruss-mediated delivery of endostatin is capable of inhibiting the Wnt signaling pathway in human colorectal cancer cells (Figs. 1 and 2; ref. 23), this inhibition is unlikely to be required for endostatin-mediated apoptosis (Figs. 3 and 4). This lack of requirement is illustrated in the data presented in Fig. 4, in which AdEndo-infected HCT116 cells undergo apoptosis without affecting the levels of β-catenin. Interestingly, endostatin-mediated apoptosis appears selective to colorectal cancer cells, as this phenomenon was not observed on a panel of breast carcinoma cell lines. Attempts to elucidate the mechanism of endostatin-mediated apoptosis showed a lack of involvement of survivin (data not shown). In addition, high-throughput experiments using the Oligo GEArray Human Signal Transduction PathwayFinder Microarray failed to highlight a specific signaling pathway activated by endostatin and were largely inconclusive (data not shown).

The intracellular effects of endostatin have been thoroughly studied in endothelial cells (45, 46). Genome-wide expression profiling coupled with reverse transcription-PCR and protein phosphorylation analysis showed that endostatin down-regulates many signaling pathways associated with proangiogenic activity and influences a large number of signaling pathways. If these results can be extrapolated to human colorectal cancer cell lines and primary desmoid tumor cells, the induction of apoptosis observed is likely to result from a complex interaction between different signaling pathways and is unlikely to be linked to the activation of a few specific pathways. Further studies will be required to clarify this mechanism of action.

The exploitation of endostatin in cancer therapy is well documented and relies essentially on its antiangiogenic effects (46). In the present report, we show that endostatin can also act directly on primary desmoid tumor cells and induces apoptosis. As endostatin acts as an autocrine/paracrine agent (data not shown), its therapeutic action is unlikely to be restricted to the infected cells and may spread well beyond the reach of the gene delivery vector. Therefore, adenovirus-mediated endostatin gene therapy may inhibit the formation of blood vessels on the cortex of the desmoid tumors and induce apoptosis in the fibroblast-like cells in the core of the tumor.

Gene therapy for desmoid tumors would probably aim at debulking the tumor before attempting surgical resection. Direct injection of therapeutic agents into desmoids under radiological guidance has been reported (47) and this route of administration could be envisaged. Even a relatively modest effect could have an important therapeutic effect by making a previously inoperable intra-abdominal tumor amenable to surgery. Therefore, based on the experimental evidences provided in this report, we advocate that adrenovirus-mediated endostatin delivery may be used in the clinical management of desmoid tumors, particularly in the context of FAP-related desmoids.

Acknowledgments

Received 4/1/2006; accepted 5/25/2006.

Grant support: Cancer Research UK.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

Assessment of Endostatin Gene Therapy for Familial Adenomatous Polyposis–Related Desmoid Tumors


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/16/8233

Cited articles

This article cites 47 articles, 22 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/16/8233.full.html#ref-list-1

Citing articles

This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/66/16/8233.full.html#related-urls

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.