Genes encoding human caveolin-1 and -2 are co-localized to the D7S522 locus (7q31.1), a known fragile site (FRA7G) that is frequently deleted in human cancers

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Abstract The $(CA)_n$ microsatellite repeat marker D7S522 is located on human chromosome 7q31.1 and is frequently deleted in a variety of human cancers, including squamous cell carcinomas of the head and neck, prostate cancers, renal cell carcinomas, ovarian adenocarcinomas, colon carcinomas, and breast cancers. In addition, D7S522 spans FRA7G, a known common fragile site on human chromosome 7. Based on these studies, it has been proposed that an as yet unidentified tumor suppressor gene (or genes) is contained within or located in close proximity to this locus. However, the identity of the candidate tumor suppressor gene at the D7S522 locus remains unknown. Here, we show that the human genes encoding caveolins 1 and 2 are contained within the same human genomic BAC clones and co-localize to the q31.1-q31.2 region of human chromosome 7, as seen by FISH analysis. In addition, we determined the intron-exon boundaries of the human caveolin-1 and -2 genes. The human caveolin-1 gene contains three exons, while the human caveolin-2 gene contains two exons. Interestingly, the boundary of the last exon of the human caveolin-1 and caveolin-2 genes are analogous, suggesting that they arose through gene duplication at this locus. $(CA)_n$ microsatellite repeat marker analysis of these caveolin genomic clones indicates they contain the marker D7S522 (located at 7q31.1), but not other microsatellite repeat markers tested. The close proximity of caveolins 1 and 2 to the D7S522 locus was independently confirmed by using a panel of MIT/Whitehead human STS markers that are known to map in the neighborhood of the D7S522 locus. As it has been previously shown that caveolin 1 possesses transformation suppressor activity (Koleske, A.J., Baltimore, D. and M.P. Lisanti (1995) Proc. Natl. Acad. Sci. USA 92, 1381–1385; Engelman, J.A. et al. (1997) J. Biol. Chem. 272, 16374–16381), we propose that the caveolin-1 gene may represent the candidate tumor suppressor gene at the D7S522 locus on human chromosome 7q31.1.

1. Introduction

Deletion of the q31 region of human chromosome 7 has been implicated in the pathogenesis of many different types of human cancers, such as oral squamous cell carcinomas [1–3], prostate carcinomas [4–7], adenocarcinoma of the stomach [8], renal cell carcinomas [9], ovarian carcinomas [10,11], colorectal carcinomas [3], mammary carcinomas [7,12–14], pancreatic carcinomas [15], and uterine leiomyomas and sarcomas [16,17]. Detection of such deletions has been performed in most cases using LOH (loss of heterozygosity) analysis, by employing specific $(CA)_n$ repeat microsatellite polymorphic markers that map to the 7q31 region. These include, but are not limited to, the following Généthon markers: D7S471; D7S480; D7S486; D7S490; D7S522; D7S523; D7S649; and D7S2456. Based on these studies, it has been proposed that an as yet unknown tumor suppressor gene resides at 7q31.1.

Among these markers, the D7S522 locus appears to be the most informative. Deletions in the 7q31.1 region are normally distributed around the D7S522 locus, defining a smallest common deleted region of ~1000 kb [3,4,10,18]. It has been reported that the D7S522 locus is the most commonly deleted region in primary breast cancers [13]. In addition, loss of D7S522 was strongly associated with systemic progression and death due to prostate cancers [4,6]. Loss of the D7S522 locus (7q31.1) also occurs in human squamous cell carcinomas [1–3], renal cell carcinomas [9], ovarian carcinomas [19], and colon carcinomas [3]. These published results are summarized in Table 1. D7S522 also spans the aphidicolin-induced fragile site FRA7G at 7q31 [9,20]. Given the usefulness of 7q31.1 and DS7522 LOH as markers for carcinogensis, many laboratories are currently searching this chromosomal region to identify a novel tumor suppressor gene that is predicted to reside at this locus. However, no genes have been previously localized to the D7S522 locus.

Here, we present conclusive evidence that the genes encoding caveolins 1 and 2 are co-localized at the D7S522 locus. As it has been reported that caveolin 1 can function as a suppressor of cell transformation in murine fibroblasts and human breast cancer cell lines [21–25], we suggest that caveolin 1 may represent the missing tumor suppressor at the D7S522 (7q31.1) locus.

2. Materials and methods

2.1. Isolation of human genomic BAC clones

Probes corresponding to the full-length cDNAs of human caveolin 1 and caveolin 2 were used to screen a human genomic BAC library (Release II; Genome Systems). Intron-exon boundaries of the human caveolin-1 and caveolin-2 genes were established using a combination of direct dideoxy sequencing of the human BAC clones and PCR analysis with primers based on the known intron-exon organization of the murine caveolin-1 and caveolin-2 genes. The presence of the coding exons of human caveolins 1 and 2 within a given BAC clone was also verified by PCR analysis.

2.2. Fluorescence in situ hybridization (FISH) analysis

Chromosomal localization of the human caveolin-1 and caveolin-2 genes was carried out in collaboration with Genome Systems. Briefly, a given human genomic BAC clone was labeled with digoxigenin dUTP by nick translation. The labeled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes (derived from PHA-stimulated human peripheral blood lymphocytes). Hybridization was carried out in a solution containing 50%

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Table 1
Reported loss of the D7S522 locus in human cancers

Tumor type	D7S522 LOH	References
Squamous cell	~16%; ~53%	[1,3]
Colon	~80%	[3]
Prostate	~83%	[4]
Renal cell	~24%	[9]
Ovarian	~73%, ~34%	[10,19]
Mammary	~83%	[13]

formamide, 10% dextran sulfate and 2×SSC. Hybridization signals were detected by incubating the hybridized slides with FITC-labeled anti-digoxigenin IgGs. Chromosomes were also counter-stained with DAPI. In double-labeling experiments, a biotin-labeled probe specific for the centromeric region of human chromosome 7 was used to confirm the localization of the caveolin-1 and caveolin-2 genes. The biotin-labeled probe was detected with Texas-Red conjugated avidin. For each caveolin genomic clone examined, a total of 80 metaphase cells were analyzed with >70% showing specific labeling.

2.3. Microsatellite and STS marker analysis

Isolated human genomic BAC clones containing caveolin genes were subjected to microsatellite marker analysis by PCR with the following 19 Généthon markers: D7S23; D7S471; D7S480; D7S486; D7S490; D7S522; D7S523; D7S525; D7S633; D7S649; D7S650; D7S655; D7S677; D7S685; D7S687; D7S1482; D7S1809; D7S2456; and D7S2847. The following nine MIT/Whitehead STS markers were also evaluated: WI-455; WI-1841; WI-3876; WI-4433; WI-5336; WI-7597; WI-7882; WI-8693; and WI-8726. Généthon and MIT/Whitehead primer pairs were obtained from Research Genetics.

Table 2
Human genomic BAC clones containing caveolin 1 and caveolin 2

Clone#	Insert size	Genes	FISH analysis
1	~80–120 kb	Cav-1 and Cav-2	7q31.1-31.2
2	~80–120 kb	Cav-1 and Cav-2	N.D.
3	~80–120 kb	Cav-1 and Cav-2	7q31.1-31.2
4	~80–120 kb	Cav-1 and Cav-2	N.D.
5	~80–120 kb	Cav-1 and Cav-2	N.D.

The presence of Cav-1 and Cav-2 genes was initially determined by colony hybridization analysis using the corresponding human cDNAs as probes. The presence of Cav-1 and Cav-2 was later confirmed by PCR analysis using primers to their known coding sequence. N.D., not determined.

3. Results

3.1. Isolation of human genomic clones encoding caveolin 1 and caveolin 2

The human cDNAs encoding caveolins 1 and 2 were used to screen a human genomic BAC library to obtain corresponding clones containing the human genes encoding caveo-

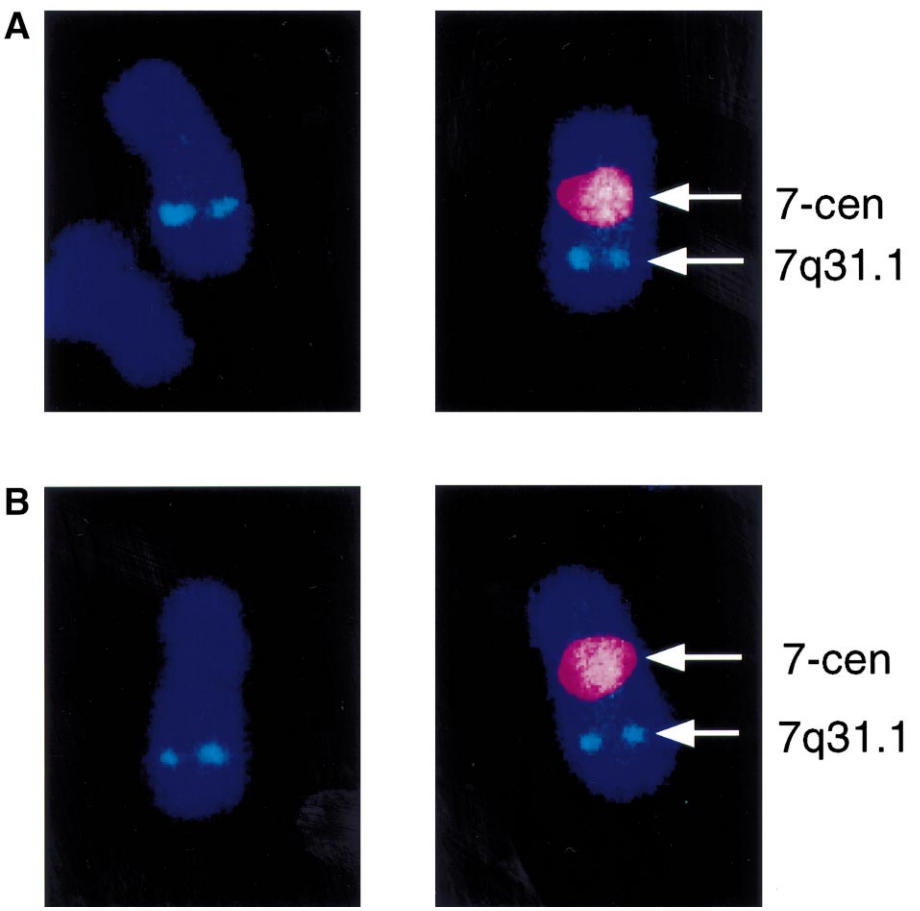

Fig. 1. Chromosomal localization of the genes encoding human caveolin-1 and caveolin-2. The chromosomal localization was determined by FISH analysis. A: Hybridization using human genomic BAC clone 1. B: Hybridization using human genomic BAC clone 3. In both cases, the hybridization signal (green) was localized to the long arm of chromosome 7 in an area which corresponds to band 7q31.1-31.2 (according to the current accepted International System for Human Cytogenetic Nomenclature). The identity of chromosome 7 was confirmed by double-labeling using a probe that is specific for the centromere of chromosome 7 (red; right panels).

A) hCav-1

Exon 1
MSGGKYVDSE

Exon 2
GHLYTVPIREQGNIYKPNNKAMADELSEKQVYDA
HTKEIDLVNRDPKHLNDDVVK

Exon 3
IDFEDVIAEPEGTHSFDGIWKASFTTFTVTKYWF
YRLLSALFGIPMALIWGIYFAILSFLHIWAVVPC
IKSFLIEIQCISRVYSIYVHTVCDPLFEAVGKIF
SNVRINLQKEI

B) hCav-2

Exon 1
MGLETEKADVQLFMDDDSYSHHSGLEYADPEKFA
DSDQDRDPHRLNSHLK

Exon 2
LGFEDVIAEPVTTHSFDKVWICSHALFEISKYVM
YKFLTVFLAIPLAFIAGILFATLSCLHIWILMPF
VKTCLMVLPSVQTIWKSVTDVIIAPLCTSVGRCF
SSVSLQLSQD

C)

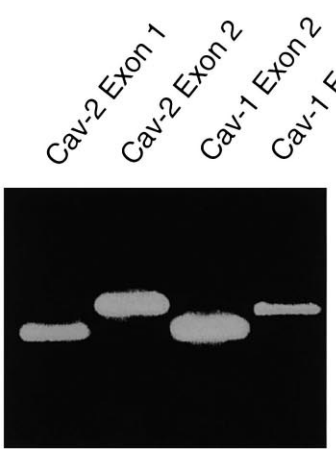

Fig. 2. Genomic organization of the human caveolin-1 and caveolin-2 genes. Exon boundaries are as indicated. A: Human caveolin-1 gene; B: human caveolin-2 gene. A similar genomic organization has been reported for the genes encoding murine caveolin 1 and caveolin 2 [54]. Note that for human caveolin 1 two amino acid residues have been changed to reflect the correct protein sequence derived from translation of numerous human ESTs. The residue at position 82 is now D, not H; and the residue at position 144 is now I, not T. These corrected residues are conserved in all mammalian caveolin-1's sequenced to date. C: The presence of the coding exons of caveolins 1 and 2 within a given BAC clone was verified by PCR using the primer pairs listed in Table 3. Human genomic BAC clone 1 served as the template for these reactions.

lins 1 and 2. Through this screening approach a total of five independent positive clones were isolated and are listed in Table 2. All five clones contain both caveolins 1 and 2. These results indicate that the genes encoding caveolins 1 and 2 must be in relatively close proximity, as the average insert size of these human BAC clones is estimated to be ~80-120 kb.

3.2. Chromosomal localization of human caveolin-1 and caveolin-2 genes

These genomic clones were next used as probes to determine the chromosomal localization of the human caveolin-1 and caveolin-2 genes by fluorescence in situ hybridization (FISH) analysis (Fig. 1).

Initial trials indicated that hybridization resulted in the specific labeling of the long arm of a group C chromosome, with the size, morphology, and banding pattern expected for human chromosome 7. The identity and exact location was determined by double-labeling experiments employing a probe that specifically labels the centromere of chromosome 7.

For the two independent clones examined, both hybridized specifically at a position that is ~55% the distance from the centromere to the telomere of 7q. This area corresponds precisely to the region 7q31.1-31.2, according to the International System for Human Cytogenetic Nomenclature. Greater than 70% of 80 metaphase cells analyzed showed specific labeling.

Searches of the NCBI Gene Map of the Human Genome indicate that other genes in the neighborhood of the 7q31-32 region include: CFTR (cystic fibrosis transmembrane conductance regulator); interferon-related protein PC4; glia-derived nexin; endothelin receptor type B-like protein; Wnt-2; PPP1R3 (protein phosphatase 1, glycogen binding regulatory subunit); the MET proto-oncogene; laminin (beta-1); filamin-2 (actin-binding protein-280); carboxypeptidase A1; and leptin (OB locus in mice).

We have previously localized the human caveolin-3 gene to chromosome 3p25 [26,27]. Interestingly, mutations within the coding region of the human caveolin-3 gene cause autosomal dominant and recessive forms of limb-girdle muscular dystrophy [26,27].

3.3. Genomic organization of human caveolin 1 and caveolin 2

Fig. 2A,B shows the intron-exon organization of the genes encoding human caveolins 1 and 2. Intron-exon boundaries were established using a combination of direct dideoxy sequencing of the human BAC clones and PCR analysis with primers based on the known intron-exon organization of the murine caveolin-1 and caveolin-2 genes. Note that the human caveolin-1 gene consists of three exons, while the human caveolin-2 gene contains only two exons. Interestingly, the last exon of both caveolin-1 and -2 genes shows virtually the same boundary, suggesting that they arose through gene duplication at this locus. The presence of the coding exons of caveolins 1 and 2 within a given BAC clone was also verified by PCR using the primer pairs listed in Table 3, and an example is shown in Fig. 2C.

3.4. Caveolins 1 and 2 are co-localized to the D7S522 locus (7q31.1)

To more precisely determine the location of the human caveolin-1 and caveolin-2 genes, we next performed microsatellite marker analysis on the corresponding human genomic BAC clones.

As 7q31.1 corresponds to a region that is frequently deleted in human cancers, numerous microsatellite markers have been used for LOH analysis of this region. Through literature

Table 3
Primer pairs used to amplify the coding regions of the human caveolin-1 and -2 genes and the expected size of their PCR products

Caveolin region	Forward primer (5′–3′)	Reverse primer (5′–3′)	Expected size (base pairs)
hCav-1 exon 2	GGACATCTCTACACCGTTCCC	CTTGACCACGTCATCGTTGAG	165
hCav-1 exon 3	ATTGACTTTGAAGATGTGATT	TTATATTTCTTTCTGCAAGTT	342
hCav-2 exon 1	ATGGGGCTGGAGACGGAGAAG	CTTGAGATGCGAGTTGAGCCG	150
hCav-2 exon 2	CTGGGCTTCGAGGATGTGATC	TCAATCCTGGCTCAGTTGCAG	339

searches, we identified the eight most common markers as follows: D7S471; D7S480; D7S486; D7S490; D7S522; D7S523; D7S649; and D7S2456. These markers, corresponding primer sequences, and the expected size of their PCR products are as indicated in Table 4.

Table 5 shows the results of this analysis. Only microsatellite marker D7S522 was detected and is present in all five caveolin genomic clones. In the case of D7S522, a PCR product of ~220 bases was observed as expected (Fig. 3). Direct dideoxy sequencing of this PCR product unequivocally confirmed its identity as microsatellite marker D7S522 (Fig. 3).

A search of The Cooperative Human Linkage Center (CHLC) Database indicated that D7S522 is located on human chromosome 7 within interval 14 (which corresponds to the 7q31.1 region). This is in accordance with our mapping results from FISH analysis. Currently, the only known gene in interval 14 is the MET proto-oncogene (also known as the hepatocyte growth factor receptor or epithelial scatter factor receptor). However, based on current physical maps of the region, the MET proto-oncogene is located ~500 kb downstream of the D7S522 locus [18].

Fig. 4 shows the location of the human caveolin-1 and -2 genes with respect to the markers D7S486, D7S522, and the MET proto-oncogene. This represents the smallest common deleted region (~1000 kb), as previously defined by LOH analysis [4,18]. Note that D7S522 is at the center of this region. Given that the average insert size of the caveolin containing BAC genomic clones is ~80–100 kb, the caveolin-1 and -2 genes must be located a maximum distance of ~100 kb upstream or downstream of D7S522. Thus, the caveolin-1 and -2 genes are at the center of this smallest common deleted region.

In addition, through this search we identified 11 other microsatellite markers that are located in interval 14 as follows: D7S23; D7S525; D7S633; D7S650; D7S655; D7S677; D7S685; D7S687; D7S1809; D7S1482; and D7S2847. However, none of these markers were detected by PCR analysis of the human genomic caveolin BAC clones (not shown).

3.5. What about STS markers located near the D7S522 locus?

We next searched the NCBI database to identify human STS markers that are located in close proximity to the microsatellite marker D7S522. Through this search, we identified nine MIT/Whitehead STS markers that map in the neighborhood of the D7S522 locus: WI-455; WI-1841; WI-3876; WI-4433; WI-5336; WI-7597; WI-7882; WI-8693; and WI-8726. These STS markers, corresponding primer sequences, and the expected size of their PCR products are described in Table 6.

Interestingly, only the STS marker WI-5336 was detected and is present in all five caveolin genomic clones. In the case of WI-5336, a PCR product of ~110 bases was observed as expected (Fig. 5). This is in accordance with current MIT/Whitehead Genome Center maps showing that WI-5336 and WI-455 STS markers are located closest to the microsatellite marker D7S522 in the following order: D7S486; WI-5336; D7S522; and WI-455. Thus, the use of these STS markers independently confirms the close proximity of the human caveolin-1 and caveolin-2 genes to the D7S522 locus.

3.6. The caveolin-1 gene is located in extremely close proximity to D7S522

In order to determine the position of the caveolin-1 and caveolin-2 genes with respect to the D7S522 locus, we performed a series of PCR reactions using primers to the coding sequences of the caveolin genes and primers to D7S522. Human genomic BAC clone 1 served as the template for these reactions. This clone contains both Cav-1 and Cav-2 genes (Fig. 2C; Table 7) and the marker D7S522 (Fig. 3).

PCR with primers to the 5′ end of Cav-1/exon 1 and the 3′ end of D7S522 yielded a strong product of ~7–10 kb in length; similar results were also obtained by PCR with primers to the 5′ end of Cav-1/exon 2 and the 3′ end of D7S522. Both of these PCR products were gel-purified and next subjected to further PCR-based mapping studies using primer pairs to detect Cav-1/exon 2, Cav-1/exon 3, Cav-2/exon 1, Cav-2/exon 2, and D7S522.

Fig. 6A shows that both of these ~7–10-kb fragments contain Cav-1/exon 2, Cav-2/exon 1, Cav-2/exon 2, and D7S522, but lack Cav-1/exon 3. Based on these studies, a first approximation map of this region was constructed and is presented in Fig. 6B. Interestingly, these results suggest that both the Cav-2 gene and D7S522 are present within the second intron of the Cav-1 gene. In support of this map, we also observed that 3 of the 5 human genomic BAC clones that we isolated

Table 4
Commonly used 7q microsatellite markers, primer sequences, and the expected size of their PCR products

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)	Expected size (base pairs)
D7S471	AGCAGCTATTATGGAATTGC	CAACATATGCAAGGTGCCTA	181–193
D7S480	CTTGGGGACTGAACCATCTT	AGCTACCATAGGGCTGGAGG	189–206
D7S486	AAAGGCCAATGGTATATCCC	GCCCAGGTGATTGATAGTGC	114–146
D7S490	CCTTGGGCCAATAAGGTAAG	AGCTACTTGCAGTGTAACAGCATTT	92–106
D7S522	GCCAAACTGCCACTTCTC	ACGTGTTATGCCACTCCC	217–229
D7S523	CTGATTCATAGCAGCACTTG	AAAACATTTCCATTACCACTG	224–240
D7S649	ATTTTGATCCCCAGCA	GCTTTATTATGTCTGTTGTATGA	275–281
D7S2456	CTGGAAATTGACCTGAAACCTT	ACAGGGGTCTCTCACACATATTA	238–252

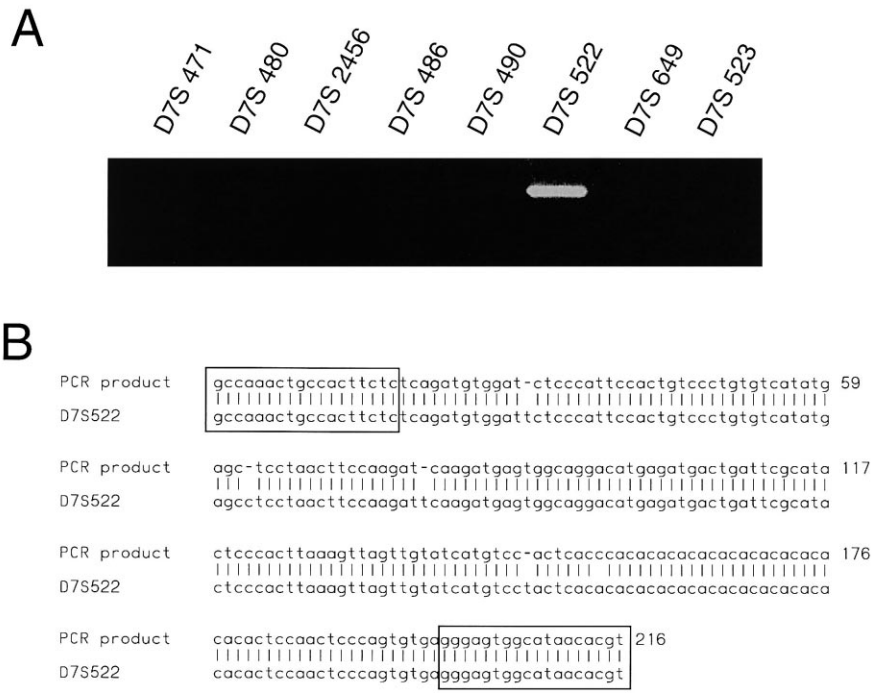

Fig. 3. Caveolins 1 and 2 are located at the D7S522 locus (7q31.1). Primers corresponding to eight Généthon microsatellite markers from the 7q31 region were used to perform PCR analysis with the human genomic caveolin BAC clones as the template. A: In the case of D7S522, a PCR product of ~220 bases was observed as expected. B: Direct dideoxy sequencing of this PCR product unequivocally confirmed its identity as the microsatellite marker D7S522. The regions contained within the PCR primers are boxed. Note that sequence of the observed PCR product and the polymorphic marker D7S522 are 97% identical at the nucleotide level. Using the D7S522 primers, identical results were obtained by direct dideoxy sequencing of the human caveolin genomic BAC clones.

contain Cav-1/exon 2, Cav-2/exon 1, Cav-2/exon 2, and D7S522, but lack Cav-1/exon 3 (Table 7).

We are currently sequencing the entire genomic BAC clone 1 (~80–100 kb) and its derivatives to obtain a more detailed map of the region.

4. Discussion

Caveolae are 50–100 nm vesicular invaginations of the plasma membrane [28,29]. It has been proposed that caveolae participate in vesicular trafficking events and signal transduction processes. Caveolin, a 21–24-kDa integral membrane protein, is a principal component of caveolae membranes in vivo [30–34].

Caveolin is only the first member of a new gene family; as a consequence, caveolin has been re-termed caveolin-1 [35]. Caveolin-2 shows the same tissue distribution as caveolin-1, co-localizes with caveolin-1, and forms a hetero-oligomeric complex with caveolin-1 in vivo [36]. In contrast, the expression of caveolin-3 is muscle-specific [37–39]. It has been proposed that caveolin family members function as scaffolding proteins [40] to organize and concentrate specific lipids (cholesterol and glyco-sphingolipids; [41–43]) and lipid-modified signaling molecules (Src-like kinases, H-Ras, eNOS and G-proteins; [41,44–48]) within caveolae membranes.

Table 5
Microsatellite marker analysis of human caveolin genomic BAC clones

Marker	Clone #				
	1	2	3	4	5
D7S471	–	–	–	–	–
D7S480	–	–	–	–	–
D7S486	–	–	–	–	–
D7S490	–	–	–	–	–
D7S522	+	+	+	+	+
D7S523	–	–	–	–	–
D7S649	–	–	–	–	–
D7S2456	–	–	–	–	–

Microsatellite repeat marker analysis was performed by PCR using the corresponding Généthon primer pairs. + indicates that a PCR product of the expected size was observed.

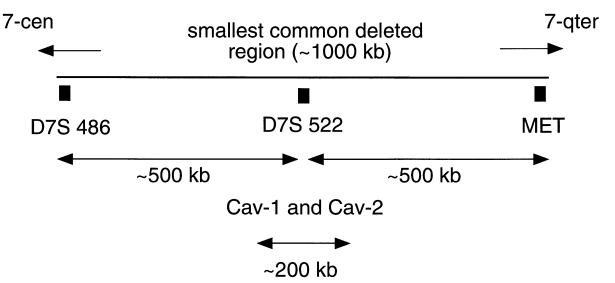

Fig. 4. Location of the human caveolin-1 and -2 genes with respect to the markers D7S486, D7S522, and the MET proto-oncogene. The smallest common deleted region (~1000 kb), as previously defined by LOH analysis, is shown [4,18]. Note that D7S522 is located at the center of this region. More specifically, D7S522 is ~500 kb downstream from the marker D7S486 and ~500 kb upstream from the MET proto-oncogene. Given that the average insert size of the caveolin containing BAC genomic clones is ~80–100 kb, the caveolin-1 and -2 genes must be located a maximum distance of ~100 kb upstream or downstream of D7S522. Thus, the position of the caveolin-1 and -2 genes lies at the center of this smallest common deleted region.

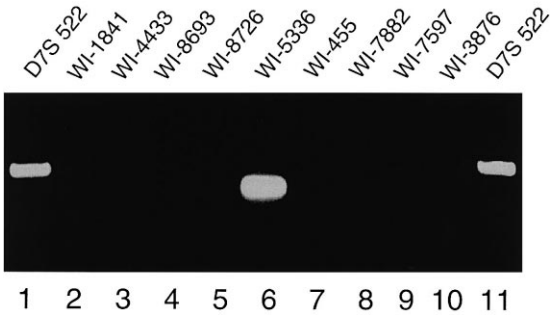

Fig. 5. STS marker analysis of the human caveolin genomic BAC clones. Primers corresponding to nine MIT/Whitehead STS markers located in the neighborhood of the D7S522 locus were used to perform PCR analysis with the human genomic caveolin BAC clones as the template. In the case of WI-5336, a PCR product of ~110 bases was observed as expected. Lanes 1 and 11 are controls using primers for amplification of D7S522 (~220 bases). Human genomic BAC clone 1 served as the template for these reactions.

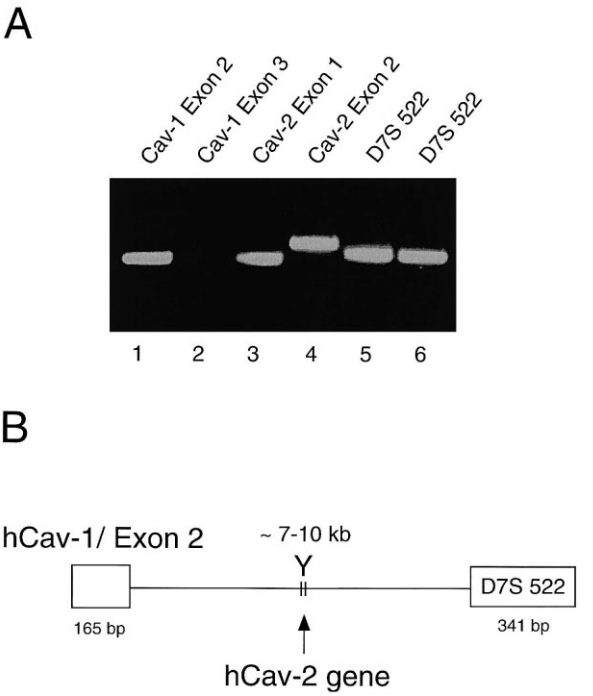

Fig. 6. Location of the human caveolin-1 gene with respect to the D7S522 locus. A: PCR with primers to the 5′ end of Cav-1/exon 1 and the 3′ end of D7S522 yielded a strong product of ~7–10 kb in length; similar results were also obtained by PCR with primers to the 5′ end of Cav-1/exon 2 and the 3′ end of D7S522 (not shown). Human genomic BAC clone 1 served as the template for these reactions. Both of these ~7–10-kb PCR products were gel-purified and next subjected to further PCR-based mapping studies using primer pairs to detect Cav-1/exon 2, Cav-1/exon 3, Cav-2/exon 1, Cav-2/exon 2, and D7S522. The expected sizes of these PCR products are listed in Tables 3 and 4. Panel A shows that both of these ~7–10-kb fragments contain Cav-1/exon 2, Cav-2/exon 1, Cav-2/exon 2, and D7S522, but lack Cav-1/exon 3. A representative gel is shown using one of the ~7–10-kb fragments as the template (lanes 1–5) or human genomic BAC clone 1 (lane 6). B: Based on the PCR analysis described in panel A, a first approximation map of this genomic region was constructed.

Caveolin-1 binding can functionally suppress the GTPase activity of hetero-trimeric G-proteins and inhibit the kinase activity of Src-family tyrosine kinases, EGF-receptor kinases, protein kinase C isoforms, and components of the p42/44 MAP kinase cascade (MEK and ERK) through a common caveolin domain, termed the *caveolin-scaffolding domain* [24,44–46,49–52]. In all cases examined, the caveolin binding motif is located within the enzymatically active catalytic domain of a given signaling molecule. For example, in the case of tyrosine and serine/threonine kinases, a kinase domain consists of 11 conserved subdomains (I–XI) [24,50,51]. The caveolin binding motif is located within conserved kinase subdomain number IX, suggesting that caveolin could function as a *general kinase inhibitor* [53]. This hypothesis has been substantiated by the observation that the caveolin scaffolding domain inhibits Src family tyrosine kinases (c-Src/Fyn), EGF-R, Neu, PKC, MEK and ERK with similar potencies [24,46,50–52]. Thus, we have suggested that caveolin may function as a negative regulator of many different classes of signaling molecules through the recognition of specific caveolin-binding motifs [49,53].

Here, we show that the genes encoding human caveolins 1 and 2 co-localize to the q31.1 region of human chromosome 7 by FISH analysis. In a previous report, we showed that the murine genes encoding caveolins 1 and 2 are co-localized to the A2 region of mouse chromosome 6 [54]. A search of the NCBI Human/Mouse Homology Map reveals that the human chromosomal region 7q31 corresponds precisely to the murine chromosomal region 6-A2. In accordance with these FISH mapping studies, we demonstrate that these human caveolin-1 and -2 genomic clones contain the $(CA)_n$ microsatellite repeat marker D7S522 and the STS marker WI-5336 that have been independently localized to the 7q31.1 region.

How does the chromosomal localization of caveolin-1 correlate with its known transformation suppressor activity? Interestingly, D7S522 spans a known common fragile site on human chromosome 7 (FRA7G) and D7S522 is frequently deleted in a variety of human cancers, including squamous

Table 6
MIT/Whitehead human STS markers in the region of D7S522, primer sequences, and the expected size of their PCR products

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)	Expected size (base pairs)
WI-455	CCTCTGATCATCTTCTGATCC	GTAAAATGGATAGATTCGGACC	250
WI-1841	CTACGTAGAAGAGAAAGGAAACGG	TTGGAGGTCTGAGTCGGAGT	208
WI-3876	GATTTCCCTAGAGCCTGGCT	GAGAGAGACCTTTCAAAATGTTCC	203
WI-4433	TGATATTAAAATGGACAAGAAATGG	GTCTTCATCATTCTCAAAACTCCA	100
WI-5336	ATAAGACCAGATCAAGGACAGACC	AACAATACACAAGCCCCTGC	112
WI-7597	CAAGTGGTTTTACAGCTACCACC	CACCCAATAGTTACAGAATATTGCC	328
WI-7882	GATGCTACAGGAATTTCAAGCC	TTATATTCTCAATGCTTTCAAAGCC	324
WI-8693	AAAACTGTGTTGGAATAAGAAAACG	AGTACCATACCTTACAGAGCTGGG	106
WI-8726	TATATCCATGGACAGGCTTGG	GAGACCATGATTTATATTGGATTGC	124

Table 7
Summary of the PCR analysis of five independent human genomic caveolin BAC clones

Clone #	Cav-1		Cav-2		Markers	
	Exon 2	Exon 3	Exon 1	Exon 2	D7S522	WI-5336
1	+	+	+	+	+	+
2	+	−	+	+	+	+
3	+	+	+	+	+	+
4	+	−	+	+	+	+
5	+	−	+	+	+	+

Primer pairs used to amplify the coding regions of human caveolin 1 and 2, and the markers D7S522 and WI-5336, as well as the expected size of their PCR products are described in Tables 3, 4 and 6. + indicates that a PCR product of the expected size was observed.

cell carcinomas, prostate cancers, renal cell carcinomas, ovarian adenocarcinomas, colon carcinomas, and breast cancers (see Section 1). Based on these studies, it has been proposed that an as yet unidentified tumor suppressor gene (or genes) is contained in close proximity to the D7S522 locus.

The unidentified tumor suppressor gene located at D7S522 (7q31.1) may be caveolin 1 given that: (i) the caveolin-1 gene is localized to this chromosomal region; (ii) caveolin-1 mRNA and protein are down-regulated during cell transformation of cultured NIH 3T3 cells, in transgenic mouse models of breast cancer, and in cell lines derived from human breast cancers [21–25]; and (iii) recombinant expression of caveolin-1 in transformed NIH 3T3 cells or cell lines derived from human breast cancers can suppress their transformed phenotype such as anchorage-independent growth in soft agar [23,25]. As many normal adult tissues express caveolin 1, it will be important to determine whether any chromosomal deletions or rearrangements of the D7S522 locus lead to a loss of caveolin-1 mRNA or protein expression during tumor formation or metastasis.

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