Chromosomal alterations in atherosclerotic plaques

Luigi Matturri [a], Alessandra Cazzullo [a], Paola Turconi [a], Anna Maria Lavezzi [a],
Pier Luigi Vandone [a], Livio Gabrielli [a], Graciela Fernández Alonso, Daniel Grana [b],
José Milei [b,c],*

[a] *Institute of Pathology, University of Milan, Ospedale Maggiore, IRCCS Milan, Italy*
[b] *CARDIOPSIS, Tucumán 2163 4to B, 1050 Buenos Aires, Argentina*
[c] *Universidad del Salvador, Buenos Aires, Argentina*

Abstract

Alterations of chromosomes 7 and 11 have been involved in the progression of atherosclerosis. Twenty-three carotid endarterectomy specimens were studied for the presence of alterations in chromosomes 7 and 11, and fibroblastic growth factor-3 (FGF-3) gene amplification. Besides classic histological stainings, immunophenotyping of cellular and vascular components and fluorescence in situ hybridization (FISH) were performed. At the caps, unstable plaques ($n = 18$) showed inflammatory infiltration of macrophages, smooth muscle cells, and T-lymphocytes. Specifically in these regions, the FISH showed varying percentages of trisomy (15/18) and tetrasomy (8/15) of chromosome 7. In four cases polisomy 7 was noted in some nuclei. Monosomy of chromosome 11 and gene amplification of FGF-3 gene was observed. The FISH of the five stable plaques and normal arterial walls showed no chromosome alterations; furthermore, chromosome 3, which is not involved in atherosclerotic progression, presented a normal ploidy of smooth muscle cells in stable and unstable plaques and normal arterial walls. In conclusion, chromosome 7 and 11 alterations and FGF-3 gene amplification are components of unstable plaques, and might contribute to the evolution of stable plaques into complicated plaques. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Chromosome 7; Chromosome 11; Fibroblastic growth factor-3, (FGF-3); Atherosclerosis; Carotid artery; Genetic alterations; Chromosomal alterations

1. Introduction

The process by which a fibrofatty plaque evolves into a fibrotic lesion, or an unstable lipid rich plaque is not clearly understood [1].

We have shown that ruptured carotid plaques present mononuclear infiltrates in the caps, shoulders and bases of the plaques. The use of specific antibodies revealed that infiltrates consist of macrophages, T-lymphocytes and scarce B-lymphocytes [2,3]. This excessive accumulation of inflammatory cells in the caps of atherosclerotic plaques is believed to be the major cause of the progression of the disease and the origin of plaque rupture [1–3].

Recently, in a paper written by our group [4], it was shown that in unstable plaques, the chromosome 7 trisomy was the most constant and significant finding. The authors speculated that the presence of a 7 extra chromosome might be correlated with an over-expression of the gene of chain A of the platelet derived growth factor (PDGF), which is mapped at pter-q22 region [5]. As a consequence, an increase of smooth muscle cell (SMC) proliferation may take place [4]. Of note, the epidermal growth factor gene is also mapped on chromosome 7 [6,7].

Trisomy 7 was found as the unique chromosome alteration in benign and malignant tumors. It was shown in cultured cells from undamaged areas of the lung [8] and kidney [9] of patients that suffered from lung and renal carcinomas, reinforcing the debate of the role of this alteration in normal and tumoral tissue, and furthermore in atherosclerosis [10].

* Corresponding author. Tel.: +54-11-49510366; fax: +54-11-49510366.

E-mail address: milei@cardioweb.net.ar (J. Milei).

It has been suggested that plaque progression involves cell deletion, proliferation and migration, and extracellular matrix synthesis and proteolysis [11,12]. These complex processes are regulated by a wide variety of factors that modulate the growth of vascular SMC and the production of mediators in the remodeling of extracellular compartments of arteries [11,12].

Chromosome instability has been suggested as the basis or consequence of increased proliferative activity of SMCs. Therefore, there appears to be a similarity between the atherosclerotic process and benign tumors [10,13]. Noteworthy is the recurring presence of trisomy 7 [4].

These observations encouraged us to investigate the influence of genetical alterations of chromosomes 7 and 11 in carotid atheromatosis progression. The gene for FGF-3 (fibroblastic growth factor-3) is located in the chromosome 11, locus 11q 13.3 (proto-oncogene FGF-3/INT2) [14]. Amplification of this gene has been reported in different types of malignant tumors [15]. However, its role has not been investigated in atherosclerosis. Therefore, alterations of chromosome 11 and FGF-3 gene might be of particular interest.

In brief, carotid endarterectomy specimens were investigated for alterations of the chromosomes 7 and 11, and amplification of the gene for FGF-3 using in situ hybridization. In this technique cellular relationships are maintained and it is possible to identify cell types expressing the gene of interest [16].

2. Methods

Carotid endarterectomy specimens were obtained from 23 consecutive patients (12 men and 11 women; mean age 65.5 years; range 43–76 years) treated by endarterectomy because of occlusive carotid disease with a lumen obstruction > 70%. Eighteen plaques were unstable and 5 were stable (see Section 3). Before surgery, all patients underwent a color echo-Doppler ultrasound examination, brain CT scan and carotid angiography. The method of endarterectomy involved a long arteriotomy with dissection of the atherosclerotic lesions in toto.

2.1. Tissue processing

Endarterectomies were fixed in 10% buffered formalin solution, and slowly decalcified in 2.5% nitric acid. All the specimens were paraffin embedded and horizontally sliced into 1 mm. segments. Paraffin blocks were serially cut at 3–5 μm thickness, and, every 20th section stained with hematoxylin and eosin and Azan. The following 15 serial sections were mounted on poly-L-lysine coated slides and used for immunostaining techniques and fluorescence in situ hybridization (FISH).

2.2. Immunocytochemistry

Immunophenotyping of cells present in carotid plaques was performed with monoclonal antibodies to identify lymphocyte subsets (T-cells, CD45RO, Biogenex; B-cells, CD20, Dako Co.; Cytotoxic/suppressor, CD8, Dako Co; Helper/inducer, OPD4, Biogenex), macrophages (CD68, Dako Co.), endothelial cells (CD31, CD34, Biogenex; Factor VIII, Ylem-Milano) and smooth muscle cell hyperplasia (HHF35, Biogenex). Detection systems used were: 1-Streptavidin biotin peroxidase or/and alkaline phosphatase (Biogenex, San Ramón, CA) and 2-EPOS (Dakopatts, Carpintería, CA). When double immunostaining was required, EPOS system, a single step with peroxidase conjugated primary antibodies was performed, followed by a second detection with streptavidin biotin alkaline phosphatase. Development of peroxidase reactions was carried out with 3, 3′ diaminobenzidine and fast red was used to visualize alkaline phosphatase reactions. Thus, the first antigen was decorated in brown and the second in bright red. Negative controls were run simultaneously with irrelevant antibodies of the same isotype.

2.3. FISH

The α-satellite DNA probe specific for the centromeric region of chromosomes 7 and 11, labelled with biotin (ONCOR Gaithersburg MD, USA) were used. The probe specific for genomic sequences, including the FGF-3 locus, was also employed (ONCOR).

Three to 5 μm thick paraffin-embedded sections were deparaffinized by two changes of xylene for 10 min each, then two changes of 100% ethanol for 5 min each. Slides were removed from ethanol and allowed to air dry. Pretreatment solution (ONCOR) preheated at 45°C was prepared before incubating the slides for 15 min. Slides were rinsed in 2 × SSC and then protein digestion with a solution of proteinase K 0.25 mg/ml at 45°C for 15 min was performed. Slides were rinsed in 2 × SSC for several s and dehydrated in 70, 80 and 95% ethanol for 1 min each and allowed to air dry.

Centromeric probe was prepared by mixing 1.5 μl of the probe with 30 μl of Hybrisol VI (ONCOR). The probe was applied to the prepared air-dried slides (15 μl) and coverslipped.

Both probes and target DNAs on the slides underwent denaturation on a 67 ± 2°C hot plate for 5 min and then overnight incubation in a pre warmed humidified chamber at 37°C.

The hybridized signals were detected by using a commercial kit (FITC avidin detection kit, ONCOR). Propidium iodide 2.5 μg/ml in antifade was used for counterstaining.

The α-satellite DNA probe specific for the centromeric region of chromosome 3, that is not involved

in atherosclerotic progression, was also used to ensure that these cells have two FISH positive signals in each nuclei.

For scoring, a Leitz Orthoplan with Ploemopak incident-light fluorescence microscope, equipped with $\times$ 40, $\times$ 100 and oil immersion objectives and ultraviolet excitation filter sets were used.

Only interphase cell nuclei with intact morphology were scored.

The FISH method was also applied to five fragments of the walls of normal arteries, which were used as controls.

In order to confirm changes in the number of chromosomes due to the orientation of the nuclei section, interphasic cytogenetic studies on isolated nuclei were performed. Nucleus extraction from the single paraffin embedded sections were processed according to Liehr et al. [17,18]. In brief, 25 µm sections of paraffin-embedded tissue were collected in glass tubes, deparaffinized, rehydrated, and covered with proteinase K solution. Fluid and nuclei were passed through a 55 µm nylon mesh and were collected in a plastic tube. The nuclei were obtained by centrifugation and the supernatant was removed. An aliquot of the suspension was put on a clean and dry slide. The slides were post-fixed in 100 ml‰ buffered formalin solution, were washed in PBS and dehydrated in alcohol. Finally, the slides were subjected to a normal FISH procedure.

3. Results

The carotid bifurcation and the first 10–15 mm. of the internal carotid were involved in all 23 cases.

3.1. Immunocytochemical findings

The characteristic feature of unstable plaques was the presence of large lipid cores with a thin fibrous cap overlying the lipid core and a band of fibrous tissue of different thickness separating the plaque from the remaining media [19]. In these cases, extensive chronic inflammatory infiltrates consisted of macrophages (CD68 +), approximately 2/3 of the total infiltration. The remaining 1/3 was made-up of T-lymphocytes and scarce B-lymphocytes. CD8 + cells predominated among T lymphocytes while CD4 + cells were very sparse. Smooth muscle cells in different quantities were always present. In five cases corresponding to stable plaques, the specimens were composed of dense fibrous connective tissue without a clear lipid core, as well as irregular masses of calcified material and scarce cellular and vascular components.

Two types of lipid cores could be observed. Avascular or mild vascularized with scarce lipid content and a lipid rich, highly vascularized one, with neoformed vessels stained with CD34 and CD31.

3.2. FISH

Varying percentages of cells with three fluorescent signals, indicating the presence of trisomy of chromosome 7, were found in 15 of the 18 'unstable plaques' (83.3%) and limited to the regions with high cellularity. Tetrasomy was observed in eight cases, and polisomy (more than four signals) were denoted in four cases (Fig. 1). Cells with locations of three hybridization signals were most frequent in all positive cases, suggesting that trisomy seven is the most common feature of unstable plaques (Table 1).

Chromosome 7 trisomy was limited to the actin smooth muscle positive cell population (HHF35).

Regarding chromosome 11 and in contrast to chromosome 7 findings, a lack of a chromosome was observed in at least 20% of cells, (Fig. 2). The gene for FGF-3 showed a marked amplification in approximately 2/3 of SMC (locus 11q13.3) (Fig. 3).

The FISH analysis of the stable plaques and normal arterial walls showed only cells with two signals per nucleus and without numerical alterations of the chromosomes 7 and 11, or alterations of the gene for FGF-3 (Fig. 4).

Concerning chromosome 3, it presented 2 FISH positive signals in each nuclei, pointing out a normal ploidy of SMC in stable and unstable plaques, and normal arterial walls (Fig. 5).

Interphasic cytogenetic studies on isolated nuclei of SMC showed the presence of trisomy, tetrasomy and polisomy of chromosome 7 and monosomy of chromosome 11 (Fig. 6), in at least 20% of the analysed nuclei 200. These data confirmed the results obtained in the tissue sections.
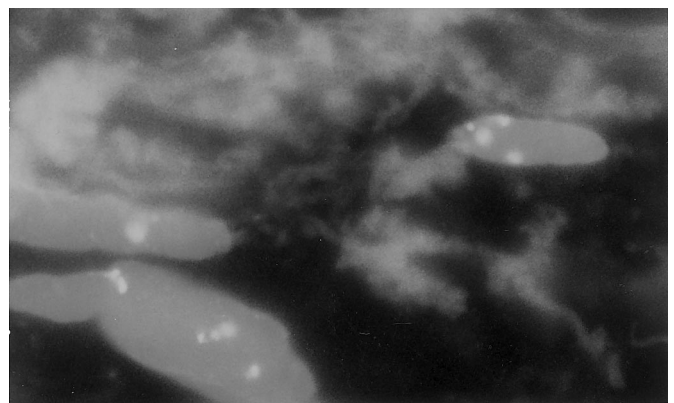

Fig. 1. FISH with the probe for chromosome 7 on paraffin-embedded section of an unstable atherosclerotic plaque. The cell nuclei show several hybridization signals (trisomy, tetrasomy and polisomy 7) ($\times$ 1000).

Table 1
Cytogenetic classification of 18 cases of human unstable atherosclerotic plaques with numerical aberrations of chromosome 7, based on FISH data from interphase cell nuclei (200 cells analyzed per case)

Case number	FISH cytogenetic classification		
	% Cells with TRISOMY 7 (3 signals/nucleus)	% Cells with TETRASOMY 7 (4 signals/nucleus)	% Cells with POLYSOMY 7 (>4 signal/nucleus)
1	20	3	3
2	19	1	–
3	30	8	–
4	25	5	–
5	18	2	2
6	15	–	–
7	37	6	–
8	10	–	–
9	47	15	4
10	20	6	–
11	25	–	3
12	33	–	–
13	25	15	–
14	19	8	–
15	22	–	–
16	–	–	–
17	–	–	–
18	–	–	–

4. Discussion

Several initiating mechanisms responsible for atherosclerosis development have been postulated including the response-to-injury theory and the theory of viral atherogenesis. The monoclonal theory of atherosclerosis postulates that the initial vascular SMC proliferative event involves the expansion of a single cell or a sub-population of cells, thus implying differences in the replicative potential of SMCs [20]. The monoclonal nature of many cancers is well-documented, and previous data analyzing mosaicism and chromosomal abnormalities in humans have demonstrated monoclonal expansions in atherosclerotic lesions [21,22]. Several lines of evidence suggest that mutation events may be involved in the development of atherosclerosis [21,22].

In this paper, it was shown that unstable atherosclerotic plaques presented a variety of chromosomal abnormalities (trisomy and tetrasomy of chromosome 7 and monosomy of chromosome 11). Since the study was performed on tissue sections, isolated cells were also used in our laboratory to confirm these results.

Additionally, the gene for FGF-3 showed a marked amplification in 2/3 of the SMC. As previously mentioned, these alterations were observed in unstable plaques, but neither in stable plaques nor normal arterial walls. On the other hand, normal disomy of chromosome 3 was observed in preserved and in abnormal arteries. This ensured that general ploidy of SMC in atherosclerosis is not being evaluated and that the data truly represents important chromosomal abnormalities for this disease.

Immunophenotypified SMCs of unstable plaques showed chromosome 7 trisomy and tetrasomy, thus identifying a clonal expansion corresponding to hyperplastic SMC.

Accordingly, two plaque SMC lines with extended life spans, pdSMC1A and pdsSMC2, were generated by the expression of the human papillomavirus (HPV)-16 E6 and E7 genes [20]. The first line may be taken to represent cells comprising an advanced atherosclerotic lesion and demonstrated several chromosomal abnormalities, most commonly −Y, +7, −13. The pdsSMC2 line may represent an earlier plaque SMC phenotype without presenting chromosomal alterations [20].

The genotype −Y, +7 is not new in the literature regarding atherosclerosis and SMCs [4,10,13]. The prevalence of +7 genotype in plaque SMCs involves the gene(s) located in this chromosome. Some of its products are PDGF A, tropoelastin, AQP1/CHIP28, nitric oxide synthase and EGF receptor [20].
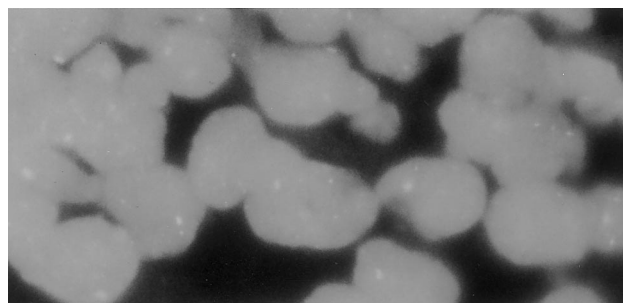

Fig. 2. Chromosome α 11. Some cells show a pair of chromosome 11, while others show a single chromosome.
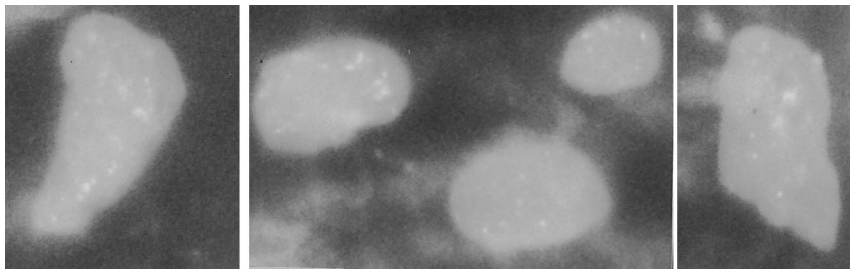
Fig. 3. FGF-3 gene amplification in smooth muscle cells. (× 1000).

Moreover, the finding of a clonal expansion, which may be considered a biological characteristic of the lesion, is the mechanism involved in the benign tumor production. As explained above, some authors [23,24] have already suggested a similarity between atherosclerotic plaque and benign tumors. Conversely, stable plaques do not present these chromosomal abnormalities.

Monosomy was found in chromosome 11, combined with a gene amplification of locus 11q13.3. Kato [25] has shown that genes coding for apolipoprotein A-1, apo C-III and A-IV are located on the long arm of chromosome 11. Genetic variation at the (apo) A-1/C-III/Aiv gene cluster on chromosome 11 has been associated with difference in occurrence of atherosclerosis and with variability in lipid levels [26].

Transcripts from FGF-3, a member of the fibroblast growth factor gene family, accumulate in cells from tumorigenic clones, but are undetectable in those from non-tumorigenic clones [27]. The physiological activity of FGF includes acting as a potent mitogen stimulating angiogenesis, brain development, cartilage formation and soft tissue repair [28]. The amplification and deletion of the FGF-3 oncogene was identified in human tumors of the breast, ovary and endometrium [15,29]. It is currently well-established that the activation of proto-oncogenes could trigger uncontrolled cell growth.

Within the last few years, a number of specific growth factors have been localized in developing lesions of atherosclerosis (PDGF, FGF, TGFbeta, IGFs, PAF). This localization was not observed in normal vessels. And because of their pleotrophic activities, a role is suggested in atherosclerosis progression [30].

Regarding FGF-3 gene amplification, as it is localized to chromosome 11, might be under-represented in the unstable plaques, taking into account that it was shown to present monosomy in a high percentage of total cells analyzed. However, FGF-3 gene amplification has been observed in cases of normal ploidy, as well as in cases of monosomy.

Chromosome 11 monosomy might be the consequence of, or contribute to, the genomic alterations involved in the atherosclerotic process. Its presence has been shown in the evolution of some neoplastic lesions [15]. For the first time, chromosome 11 monosomy is described to be related to the atherosclerotic plaque.

Pathological accumulation of cells may be due to migration of macrophages and T lymphocytes and to SMC proliferation [19]. It is now well-established that artery injury triggers excessive cell migration and proliferation and these events are considered a major cause of plaque lesion [11].

Cell accumulation in caps of plaques is the result of cell migration, growth, and death [31]. Accordingly, apoptosis was frequently found ($10.8 \pm 1\%$) in the fibrous caps of the internal layers of the lipid cores of unstable plaques where an outstanding hyperplasia of SMC and macrophages was evident [32].

On the other hand, extensive SMC proliferation is considered a hallmark of early atherosclerosis [33]. This proliferation is stimulated by different factors like cytokines and PDGF [5,6].

These findings do support the hypothesis that genetic instability may contribute to neointimal lesion. Further investigation is therefore necessary to clarify whether the predominant cytogenetic abnormalities observed in the present study contributed to the outgrowth of this population or are the consequence of other selective stimuli.

Mechanisms of atherogenesis and plaque rupture are complex processes including gene alterations, immune mediated cell changes, and alteration of growth and proliferation pathways due to various factors.

The therapeutic goals now focus on the reduction of plaque progression and prevention of plaque instability and rupture. The molecular understanding of these processes related to cell migration, proliferation and death, opens new strategies for the preventive treatment of these lesions. Interruption of growth factor activity or other regulatory mechanisms may prevent the onset, progression, and development of further complications and may even induce plaque regression.

Fig. 5. Chromosome 3 presented two FISH positive signals pointing out a normal ploidy of SMC ($\times$ l000).

Fig. 6. FISH on isolated nuclei of SMC from unstable plaques showing monosomy of chromosome 11 ($\times$ 1000).
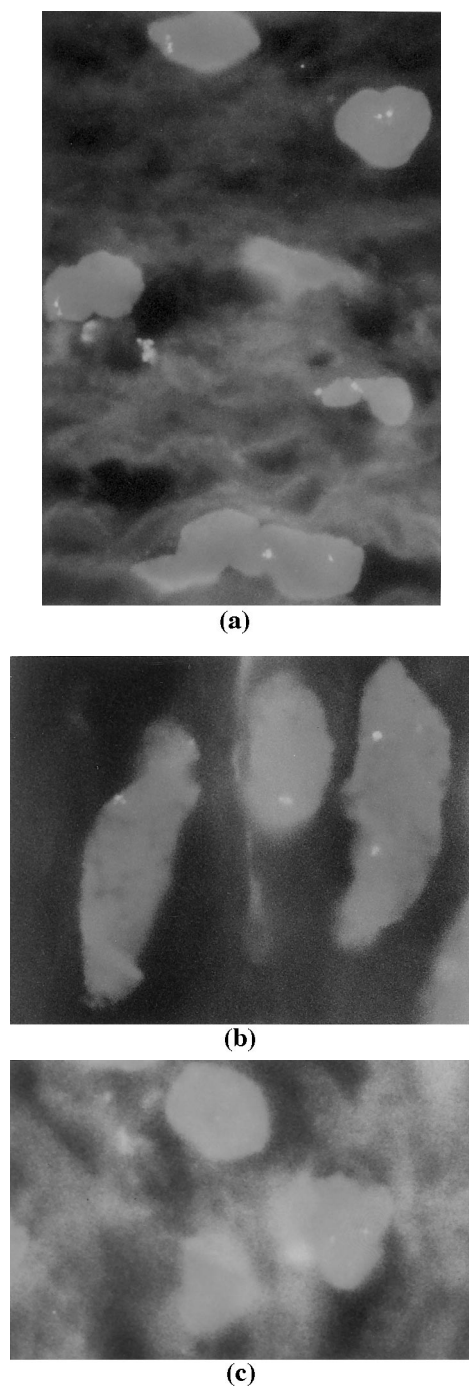

(a)

(b)

(c)

Fig. 4. No numerical alterations of the chromosome 7 (A) and 11 (B), and no alterations of the gene for FGF-3 (C) were observed in stable plaques and normal arterial walls ($\times$ 1000).
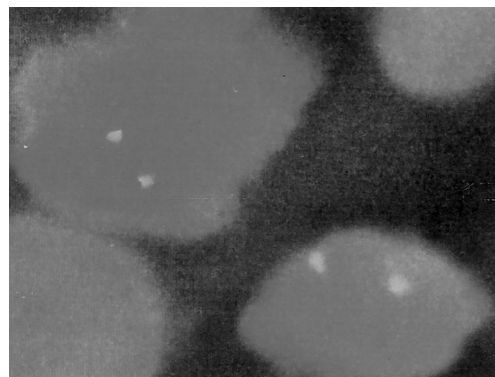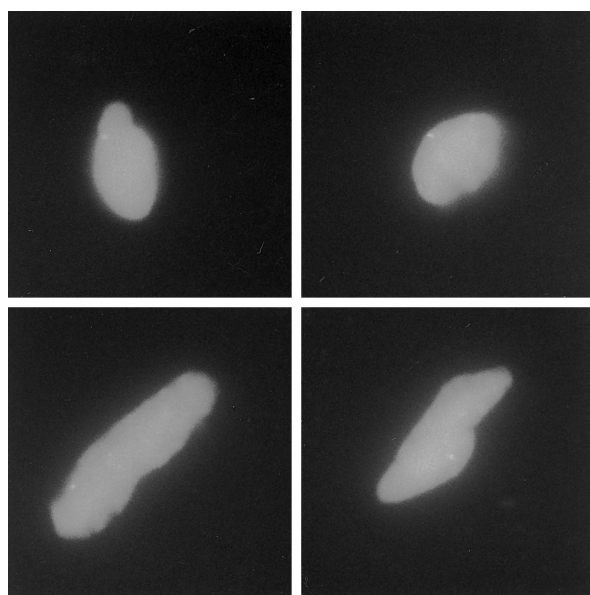
References

[1] Newby AC, Libby P, van der Wal AC. Plaque instability-the real challenge for atherosclerosis research in the next decade? Cardiovasc Res 1999;41:321–2.

[2] Milei J, Parodi JC, Fernández AG, Barone A, et al. Carotid atherosclerosis: immunocytochemical analysis of the vascular and cellular composition in endarterectomies. Cardiologia 1996;41:535–42.

[3] Milei J, Parodi JC, Fernández AG, Barone A, Grana D, Matturri L. Carotid rupture and intraplaque hemorrhage: immunophenotype and role of cells involved. Am Heart J 1998;136:1096–105.

[4] Matturri L, Cazzullo A, Turconi P, Lavezzi AM. Cytogenetic aspects of cell proliferation in atherosclerotic plaques. Cardiologia 1997;42:833–6.

[5] Yu SM, Hung LM, Lin CC. cGMP-elevating agents suppress proliferation of vascular smooth muscle cells by inhibiting the activation of epidermal growth factor signaling pathway. Circulation 1997;95:1269–77.

[6] Barret TB, Benditt EP. Platelet-derived growth factor gene expression in human atherosclerotic plaques and normal artery wall. Proc Natl Acad Sci USA 1998;85:2810–4.

[7] Scott J. Oncogenes in atherosclerosis. Nature 1987;325:574–5.

[8] Lee JS, Pathak S, Hopwood V, et al. Involvement of chromosome 7 in primary lung tumor and nonmalignant normal tissue. Cancer Res 1987;47:6349–52.

[9] Kovacs G, Brusa P. Clonal chromosome aberrations in normal kidney tissue from patients with renal cell carcinoma. Cancer Genet Cytogenet 1989;37:249–50.

[10] Vanni R, Cossu L, Licheri S. Atherosclerotic plaque as a benign tumor? Cancer Genet Cytogenet 1990;47:273–4.

[11] Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. New Engl J Med 1994;330:1431–8.

[12] Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell. Science 1973;180:1332–9.

[13] Casalone R, Granata P, Minelli E, et al. Cytogenetic analysis reveals clonal proliferation of smooth muscle cells in atherosclerotic plaques. Hum Genet 1991;87:139–43.

[14] Ibukiyama CH. Angiogenesis: angiogenic therapy using fibroblast growth factors and vascular endothelial growth factors for ischemic vascular lesions. Jpn Heart J 1996;37:285–300.

[15] Schmitt JF, Susil BJ, Hearn MT. Aberrant FGF-2, FGF-3, FGF-4 and C-erb-B2 gene copy number in human ovarian, breast and endometrial tumours. Growth Fact 1996;13:19–35.

[16] Wilcox JN. Analysis of local gene expression in human atherosclerotic plaques by in situ hybridization. Trends Cardiovasc Med 1991;1:17–24.

[17] Liehr T, Grehl H, Rautenstrauss B. FISH analysis of interphase nuclei extracted from paraffin-embedded tissue. Trends Genet 1995;11:377–8.

[18] Liehr T, Uwe C, Erich G. Nucleus extraction from single mounted tissue sections: genetic analysis. Biomol Eng 1999;15:65–9.

[19] Newby AC, George SJ. Proliferation, migration, matrix turnover, and death of smooth muscle cells in native coronary and vein graft atherosclerosis. Curr Opinion Cardiol 1996;11:574–82.

[20] Bonin LR, Madden K, Shera K, et al. Generation and characterization of human smooth muscle cell lines derived from atherosclerotic plaque. Arterioscler Thromb Vasc Biol 1999;19:575–87.

[21] McCaffrey TA, Du B, Consigli S, et al. Genomic instability in the type II TGF-β1 receptor gene in atherosclerotic and restenotic vascular cells. J Clin Invest 1997;100:2182–8.

[22] Hatzistamou J, Kiaris H, Ergazaki M, Spandidos DA. Loss of heterozygosity and microsatellite instability in human atherosclerotic plaques. Biochem Biophys Res Commun 1996;225:186–90.

[23] Benditt EP, Benditt JM. Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci USA 1973;70:1753–6.

[24] Pearson TA, Dillman JM, Solez K, Heptinstall RH. Clonal characteristics in layers of human atherosclerotic plaques. Am J Pathol 1978;93:1993.

[25] Kato H. Apolipoprotein A I-C III-A IV deficiency. Nippon-Rinsho 1994;52:3253–6.

[26] Carrejo MH, Sharret R, Patsch W, Boerwinkle E. No association of apolipoprotein A-IV codon 347 and 360 variation with atheroesclerosis and lipid transport in a sample of mixed hyperlipidemies. Genet Epidemiol 1995;12:371–80.

[27] Galdemard C, Brison O, Lavialle C. The proto-oncogene FGF-3 is constitutively expressed in tumorigenic, but not in non-tumorigenic, clones of a human colon carcinoma cell line. Oncogenes 1995;10:2331–42.

[28] Ibikiyama C. Angiogenesis: angiogenic therapy using fibroblast growth factors and vascular endothelial growth factors for ischemic vascular lesions. Jpn Heart J 1996;37:285–300.

[29] Esteller M, Garcia A, Martinez I, Palones JM, Cabrero A, Reventos J. Detection of c-erbB-2/neu and fibroblast growth factor-3/INT-2 but not epidermal growth factor receptor gene amplification in endometrial cancer by differential polymerase chain reaction. Cancer 1995;75:2139–46.

[30] Raines EW, Ross R. Multiple growth factors are associated with lesion of atherosclerosis: specificity or redundancy? Bioessays 1996;18:271–82.

[31] Wei GL, Krasinski K, Kearney M, Isner JM, Walsh K, Andres V. Temporally and spatially coordinated expression of cell cycle regulatory factors after angioplasty. Circ Res 1997;80:418–26.

[32] Matturri L, Cazzullo A, Turconi P, Roncoroni L, Grana D, Milei J. Inflammatory cells, apoptosis and Chlamydia pneumoniae infection in atherosclerosis, Int J Cardiol (in press, 2000).

[33] Ross R. The pathogenesis of atherosclerosis: a perpective for the 1990's. Nature 1990;362:801–9.