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Abnormal Behavior of Cultured Fibroblasts From Nodule and Nonaffected Aponeurosis of Dupuytren's Disease

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Vimentin-positive, desmin-negative cells were established in culture from the nodule and from apparently normal palmar aponeurosis of a patient with Dupuytren's disease and compared with normal human embryonic and adult fibroblasts or sarcomatous cells. Cells from the nodule display in vitro biological properties that are intermediate between those expressed by normal fibroblasts and sarcoma cells or cells from the nodule transformed with SV40 virus. Thus, they represent an interesting in vitro model of partially transformed human cells. This behavior is not evolutive and justifies the classification of Dupuytren's disease among the benign mesenchymal tumors. The production of high level of plasminogen activator probably explains the local reactive pathology, and could act as a mitogenic stimulus for the proliferation of the nodule itself. Cultures derived from the apparently normal palmar aponeurosis show some but not all the abnormal growth properties of cells from nodules; this may help to explain the onset of local recurrences. Our results suggest that Dupuytren's disease is not strictly local and limited to the nodules, but affects, at least partially, the whole aponeurosis. Dupuytren's nodules could be considered as a model of tumor progression in a benign situation.

Dupuytren's contracture is a disease that arises in the palmar aponeurosis and causes the progressive irreversible contracture of one or more digits (Dupuytren, 1831–1832). Accepted predisposing factors include aging, race (Caucasian), sex (male), and heredity (Larsen, 1966).

Characteristic of the disease is the presence of nodules that develop in the palmar aponeurosis (Dupuytren, 1831–1832) and that have been classified among the fibromatoses (Enzinger et al., 1970). The nodule seems to play a central role in the pathogenesis of the contracture (Gabbiani and Majno, 1972; Larsen, 1966; Luck, 1959; Skoog, 1948); it has been reported that the administration of cytostatics during the initial phases of the diseases causes the nodules to disappear and the contracture to stop or to regress (Aron, 1968). Although no case of metastasis has ever been reported, local recurrence is rather common after surgical excision (Larsen, 1966).

Electron microscopic studies have revealed several peculiar structural features of the cells of the nodules, which have been classified as myofibroblasts (Gabbiani and Majno, 1972). However, their exact origin is not known. Most authors agree that they derive from fibroblasts normally present in the aponeurosis (Gabbiani and Majno, 1972; Larsen, 1966; Luck, 1959; Skoog, 1948), but it has also been proposed that they may derive from perivascular elements (Larsen, 1966).

The purpose of this report is to describe cultured cells derived from: (a) one nodule, and (b) the "apparently normal" aponeurosis of the same patient and to compare the characteristics of these cells with those of cultured normal human embryonic and adult fibroblasts, of human sarcomatous cells, and the cells from the nodule transformed by SV40 virus.

The choice of human embryonic cells is due to the fact that these cells display higher proliferative potentials in comparison with adult ones (Hayflick and Moorhead, 1961; Macieira-Coelho et al., 1966; Pfeffer et al., 1976; Vincent and Huang, 1976), so that they constitute a useful intermediate between adult and sarcoma cells.

MATERIALS AND METHODS

Cells and culture procedures

The origin and the characteristics of the normal human embryonic (964-S) and adult (NMS_1) fibroblastic cell lines have been detailed elsewhere (Azzarone and Macieira-Coelho, 1982; Azzarone et al., 1981a). The human sarcomatous cell line KHOS/NP (Rhim et al., 1975)

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was kindly donated by Dr. J.S. Rhim (NIH, Bethesda, MD). The BMS7 cell line was derived from the normal skin of a 45-year-old donor and established by ourselves in culture as previously described (Azzarone et al., 1976).

Dupuytren's nodule and the apparently normal aponeurosis from a 52-year-old man were dissected into small pieces and handled for cell culture according to a previously described technique (Azzarone et al., 1976).

Initial cellular migration occurred within 36 hours from explantation around 95% of the explants derived from the nodule (DUP-N); it consisted of fibroblastic-like cells. First appearance of fibroblastic cells around aponeurosis explants (DUP-A) could be observed only 120 hr after explantation. This delay in the migrating activity could be related to the previous observation that practically all cells of the nodule have already developed in vivo the structures responsible for movement and contraction, while most cells of the surrounding tissue appear in vivo to be deprived of such a machinery (Gabbiani and Majno, 1972; for review see Rungger-Brändle and Gabbiani, 1983). We cannot exclude, however, that the delay in migrating activity is due to differences in the total cell population between the nodule and the aponeurosis.

Thereafter, sustained proliferation of fibroblastic cells occurred, and confluency was reached within 15 days of explantation for the cultures derived from the nodule, and within 30 days for the cultures derived from the aponeurosis.

When the cells from the primary cultures reached confluency, they were trypsinized and subcultivated at 1:2 split ratio. The same split ratio was used to maintain the cultures until their extinction. Phase II (PDLS 1–24) cultures were subcultivated every 6 to 7 days; this subculture interval increased progressively to 12 days in senescent cells. Cells were fed with Eagle's minimum essential medium supplemented with 10% fetal calf serum and 16 μg/ml gentamicin. The nutrient medium was left unchanged between each subcultivation. The culture protocol used is that described by Hayflick and Moorhead (1961). Cell counts were performed after trypsinization with Coulter counter.

The population doublings were calculated by the evaluation $\log_2 (N/N_0)$ where N is the final cell number and N_0 the initial cell number.

The total number of cell produced during a life span was calculated by adding the difference at each passage between the number of cells reached at confluence and the cells inoculated at subcultivation.

Cultures were routinely checked for mycoplasma contamination by orcein staining and uridine-uracil incorporation (Schneider et al., 1974), and no mycoplasmas were detected.

Resting 964S, NMS_1, BMS7, DUP-A, and DUP-N cultures were analyzed for the different biological assays between the 3rd and the 12th passage, when they were all practically at the same stage in their culture history.

SV40 transformation

DUP-N cells on th 8th passage were infected with SV40 virus at 10 PFU × cell. Transformation efficiency was determined by following the progressive increase of antigen T-positive cells. Four subcultures after infection more than 90% of the cells were positive in indirect immunofluorescence assay for the viral T antigen. At this time the SV40-infected cultures (DUP-N-SV40) displayed a transformed morphology characterized by loss of contact inhibition, criss-cross piling up, and increased refractility. Radioimmunoprecipitation assay showed the presence of 94 Kd large T antigen, as well as the 53-Kd transformation associated protein (Melero et al., 1979). The biological characterization of DUP-N-SV40 cultures was performed between the 15th and the 17th subculture. DUP-N-SV40 cultures were subcultured twice per week with the same protocol employed for DUP-N cells.

Growth kinetics

Confluent cultures were trypsinized, suspended in growth medium, and subcultured into 30-mm plastic petri dishes at 1:16 split ratio. Each day after seeding, two cultures were trypsinized, and the cells were counted with a Coulter counter until no further increase was observed without medium change. The counts in duplicate dishes differed by less than 10% throughout the experiment. To determine DNA synthesis, [^{3}H]thymidine (The Radiochemical Center, Amersham, England) was added to duplicate cultures in a final concentration of 0.1 μCi/ml (sp. act. 2 Ci/mmol).

Chromosome number

A standard procedure for air-dried preparation was used for chromosome number evaluation (Rothfels and Siminovitch, 1958).

Interferon sensitivity

Mouse interferon β was prepared from the brains of IC or Swiss mice inoculated intracerebrally with West Nile virus (Gresser et al., 1969). Cell sensitivity to the antiviral activity of interferon was tested by the method of inhibition of the cytopathic effect (CPE) of 100 $TCID_{50}$ of vesicular stomatitis virus (VSV) in cells grown as monolayer in microtest II plastic trays as described previously (Gresser et al., 1969). Titers are expressed as the reciprocal of the highest dilution giving approximately 50% protection of the cell sheet.

Evaluation of fibrin clot retraction (FCR)

FCR evaluation was performed according to a previously described technique (Azzarone et al., 1981b,c).

Formation of spontaneous aggregates in suspension

Confluent cultures were trypsinized: 2×10^5 cells in 2 ml of MEM 20% FCS were seeded onto a 1% agarose layer in MEM 20% FCS according to Steuer et al. 1977). Twenty-four hours later, the plates were scored for presence of aggregates in suspension and photographed.

Growth in soft agar

Colony formation in soft agar was performed by seeding 2×10^5 cell/30-mm petri dishes according to a previously described technique (McAllister et al., 1967). Cultures were scored for colony formation 14 days later.

Colony formation on epithelial sheet

Confluent monolayers of the normal human embryonic epithelial cell line T74 (Owens et al., 1976) were prepared in 25-cm^2 plastic flasks. Cultures to be tested were trypsinized and seeded at 10^3 cells per flask, and

the medium was changed twice a week for 21 days, fixed in methanol and stained with May-Grünwald Giemsa (Smith et al., 1976).

Growth in low serum concentration

Confluent cultures were trypsinized, resuspended in MEM supplemented with 2% FCS, and seeded into 25-cm^2 plastic flasks at 3×10^5 cells/flask. Cultures were refed twice a week. Fifteen days later, the cultures were trypsinized and counted. The efficiency was expressed as number of population doublings (PD).

Instability of postconfluent saturation density

Confluent sister cultures to those used in growth kinetics experiments were trypsinized, counted, and divided into two groups. In one, each culture was subcultivated into two new bottles (1:2 split ratio); in the other, each culture was subcultivated into one new bottle (1:1 split ratio) (Azzarone and Macieira-Coelho, 1982; Azzarone et al., 1980, 1981a).

Production of plasminogen activator (PA)

Confluent cultures were rinsed three times with EMEM and refed overnight with medium without FCS. On the day after, the presence of intracellular PA was checked according to a quantitative assay, which was carried out in multiwell tissue culture dishes coated with [^{125}I]fibrinogen (100,000 cpm per well) as described elsewhere (Failly-Crépin et al., 1981). Human plasminogen and fibrinogen were purified following methods previously described (Unkeless et al., 1973). A unit of PA activity is defined as the activity that solubilizes 5% of initial radioactivity in 1 hr after correction for background using a substrate of 10 $\mu g/cm^2$; the specific activity is expressed in unit per 10^6 cells/hr. The molecular species of PA were determined by separation on polyacrylamide SDS electrophoresis on a molecular weight basis (Granelli-Piperno et al., 1977).

Immunofluorescent assays

Confluent cultures were trypsinized, suspended in growth medium, and seeded onto glass coverslips. Forty-eight hours later the coverslips were rinsed three times with MEM without FCS and processed for immunofluorescent studies for distribution of pericellular fibronectin, actin, vimentin, and desmin cytoplasmic filaments.

Antifibronectin (Zardi et al., 1980), actin (Gabbiani et al., 1977), vimentin (Gabbiani et al., 1982), and desmin (Gabbiani et al., 1982) antibodies were obtained and utilized as previously described (Gabbiani et al., 1977, 1982; Zardi et al., 1980). Monoclonal antibodies against human fibronectin (Zardi et al., 1980) were supplied by Dr. L. Zardi (I.S.T., Genoa, Italy).

RESULTS

Growth characteristics

Figure 1 illustrates the maximal cell densities found at each subculture from the first subculture up to extinction in DUP-N and DUP-A cultures. The two curves are very similar and are of the type previously found (Hayflick and Moorhead, 1961) for normal human fibroblasts, i.e., the density declined progressively through the life span and fell rapidly during the last four to five subcultures. The calculation of the cumulative number of population doublings and of the total number of cells produced (Table 1) confirmed the identity of the in vitro growth potential exhibited by the two cell lines. On the contrary, the total number of the cells produced after

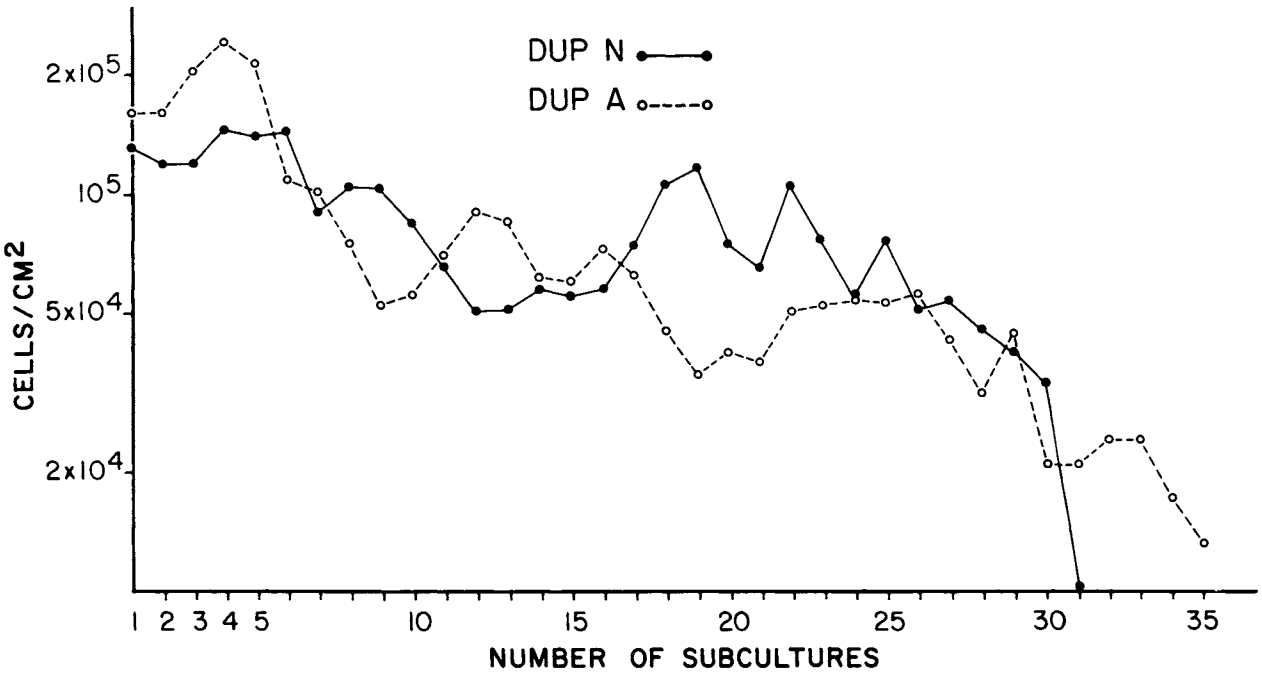

Fig. 1. Maximal cell densities found at each subculture in DUP-N (●—●) and DUP-A (○– –○) cultures.

TABLE 1. Growth properties of normal human skin embryonic (964-S) and adult (NMS_1 and BMS7) fibroblasts, human fibroblasts from a Dupuytren's nodule (DUP-N) and from the apparently normal aponeurosis (DUP-A) of a patient with Dupuytren's disease, a human sarcoma cell line (KHOS/NP) and human fibroblasts from a Dupuytren's nodule transformed with SV40 Virus (DUP-N-SV40)

Cell line	964–S	NMS_1	BMS7	DUP-A	DUP-N	DUP-N-SV40	KHOS-NP
Life span (number of PDs)	45	41	16	30	28	32 up to now	infinite
Total number of cells produced	NT	NT	NT	27.3×10^6	27.9×10^6	49×10^6 [2]	NT
Cell synthesizing DNA at the plateau phase	2.5%	1%	2%	1.5%	2%	54%	42%
Interferon sensitivity (antiviral titer)[1]	> 64000	NT	NT	> 64000	> 64000	600	800
Fibrin clot retraction	100%	100%	100%	100%	100%	< 1%	< 1%
Formation of spontaneous aggregates in suspension	Negative	Negative	Negative	Negative	Positive	Positive	Positive
Growth in soft agar (number of colonies/Petri dish)	0	0	0	0	80	1600	6000
Colony formation on human epithelial sheet	0	0	0	1%	1%	15%	70%
Number of PDs performed in 2% FCS	1	0	0	2	2	4	3
Response to 1:1 split	− 3%	− 3%	+ 3%	+ 59%	+ 66%	NT	NT
Production of plasminogen activator	Negative	Negative	Negative	Negative	$1.2U \times 10^6$ cells/hr	$5.3U \times 10^6$ cells/hr	$1.33U \times 10^6$ cells/hr

[1]Reciprocal dilution.
[2]After 28 PDs.

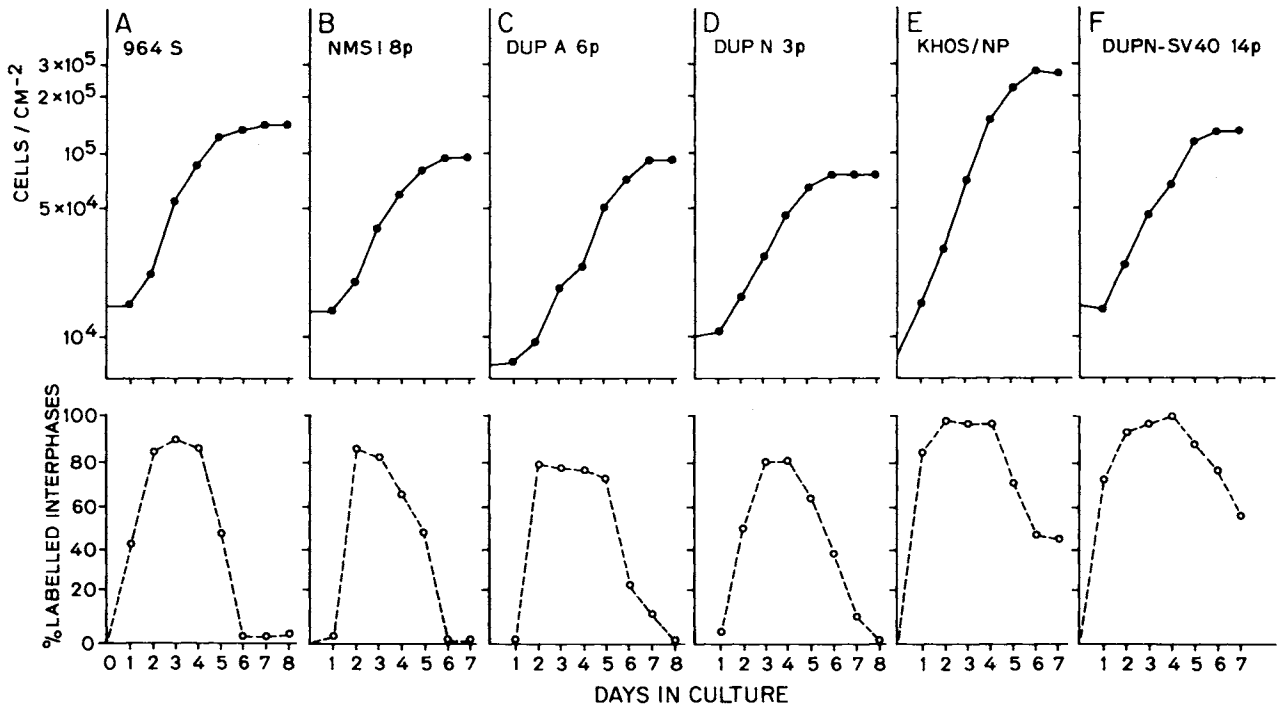

Fig. 2. Cell counts plotted semilogarithmically (●——●) and percentage of labeled interphases plotted arithmetically (○——○) on different days after subcultivation of 964–S (A), NMS_1 (B), DUP-A (C), DUP-N (D), KHOS/NP (E), and DUP-N-SV40 (F) cultures.

the same number of doublings by DUP-N-SV40 cultures was almost the double than that found in the nontransformed counterpart. This shows that the SV40-transformed cells have acquired an increased growth potential.

Growth kinetics

We have analyzed daily cell growth and DNA synthesis in normal human embryonic (964-S) and adult (NMS_1) skin fibroblasts, in a human sarcoma cell line (KHOS/NP), and in DUP-N- and DUP-A-SV40 cultures. These studies were conducted to characterize the relationships between growth fraction and contact inhibition of cell division. Eighteen hours after seeding (Fig. 2A), 964-S cultures showed a lag phase with no increase in cell number and a discrete percentage (43%) of cells synthesizing DNA. On the second, third, and fourth days after subcultivation, the cultures entered the exponential growth phase. The highest percentage of cells synthesizing DNA (80%) was obtained on the second, third, and

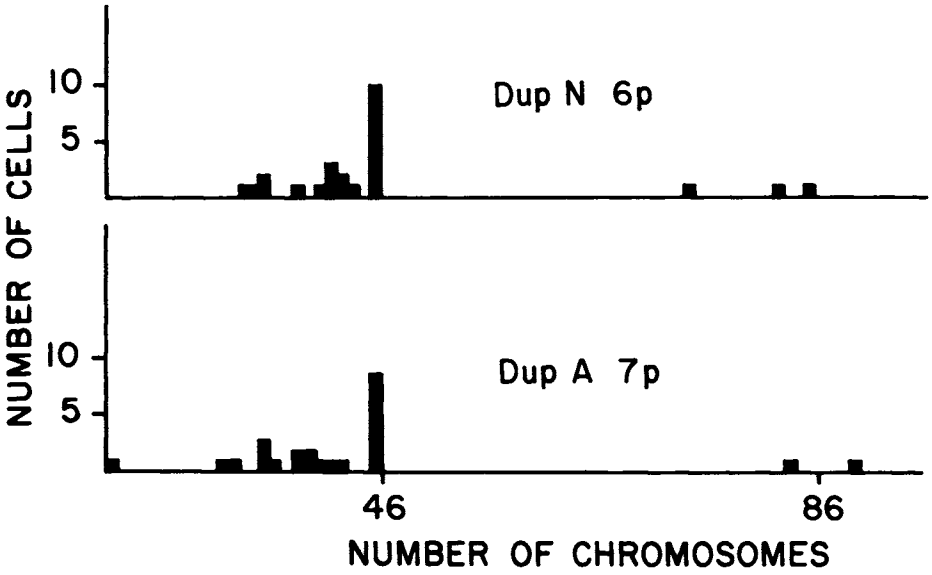

Fig. 3. Chromosome distribution in DUP-N (6p) and DUP-A (7p) cultures.

fourth day, while on the fifth day it decreased to 45%. On the sixth day, the cell number did not increase (1.5 $\times$ 10^5/cm^2), and DNA synthesis fell to low levels.

The growth curve of the sarcoma cell line is presented in Figure 3E. In this culture, the cell number was already significantly increased and more than 80% of the cells had entered DNA synthesis 18 hr after seeding. The final saturation densities reached were double (3 $\times$ 10^5 cells/cm^2) those exhibited by normal embryonic fibroblasts seeded at a similar initial inoculum. Even in these overcrowded culture conditions, more than 40% of the cells were able to synthesize DNA on the sixth and seventh day after subcultivation.

Adult NMS_1 (Fig. 2B), DUP-A (Fig. 2C), and DUP-N (Fig. 2D) showed growth kinetics very similar to those of normal embryonic cells, the percentage of cells synthesizing DNA during the lag phase was, however, very low. The final cell densities reached were in the same range for DUP-A and DUP-N and were lower (Table 1) when compared with those expressed by embryonic 964-S cells. DUP-N-SV40 cells (Fig. 2F) behaved similarly to sarcoma cells, since during the lag phase 70% of cells entered DNA synthesis, and at the plateau phase more than 50% of cells were still able to synthesize DNA. The final cell density was 70% higher than that found in DUP-N cultures.

Chromosome number

DUP-N and DUP-A cultures exhibited identical chromosome distribution: i.e., modal number of 46 chromosomes; more than 50% of the metaphases analyzed were however hypodiploid (Fig. 3).

Interferon sensitivity

It has been recently reported that a decreased sensitivity to the antiviral effect of interferon is associated with the in vitro transformation of murine cells (Azzarone et al., 1981b; Brouty-Boyé et al., 1979). Analysis of Table 1 shows that this behavior can be extended to a human sarcoma line and to DUP-N-SV40 cells. Indeed, normal cell (964-S) as well as DUP-N and DUP-A cultures showed a very high sensitivity (> 64.000), while KHOS/NP (800) and DUP-N-SV40 (600) cells appeared much more resistant.

Evaluation of FCR

It has been recently shown that normal fibroblasts from different species are able to induce high level of FCR, while their transformed counterparts loose this property (Azzarone et al., 1981b,c; Dolfini et al., 1976).

Analysis of Table 1 illustrates that normal human fibroblasts (964-S and NMS_1) as well as DUP-N and DUP-A cultures display maximal levels of this activity (100%), while KHOS/NP and DUP-N-SV40 cells have completely lost this cell function.

Formation of spontaneous aggregates in suspension

It has been recently shown that normal fibroblasts are unable to form spontaneous aggregates in suspension when they are seeded on a hard agar layer, while transformed cells are able to do so (Steuer et al., 1977). Human normal fibroblasts (964-S, NMS_1, and BMS7) (Fig. 4A) and DUP-A cells (Fig. 4B) appeared 24 hr after seeding as an almost monocellular suspension, while DUP-N cells (Fig. 4C) formed a great number of aggregates, whose size was, however, smaller than that of KHOS/NP cells (Fig. 4D). DUP-N-SV40 produced aggregates similar in number and size to those produced by KHOS/NP cells (data not shown).

Growth in soft agar

The cultures were left for 2 weeks in agar medium, and the number of colonies was counted; only aggregates with more than 20 cells were considered as colonies. As summarized in Table 1, human normal fibroblasts (964-S, NMS_1, and BMS7) and DUP-A cells

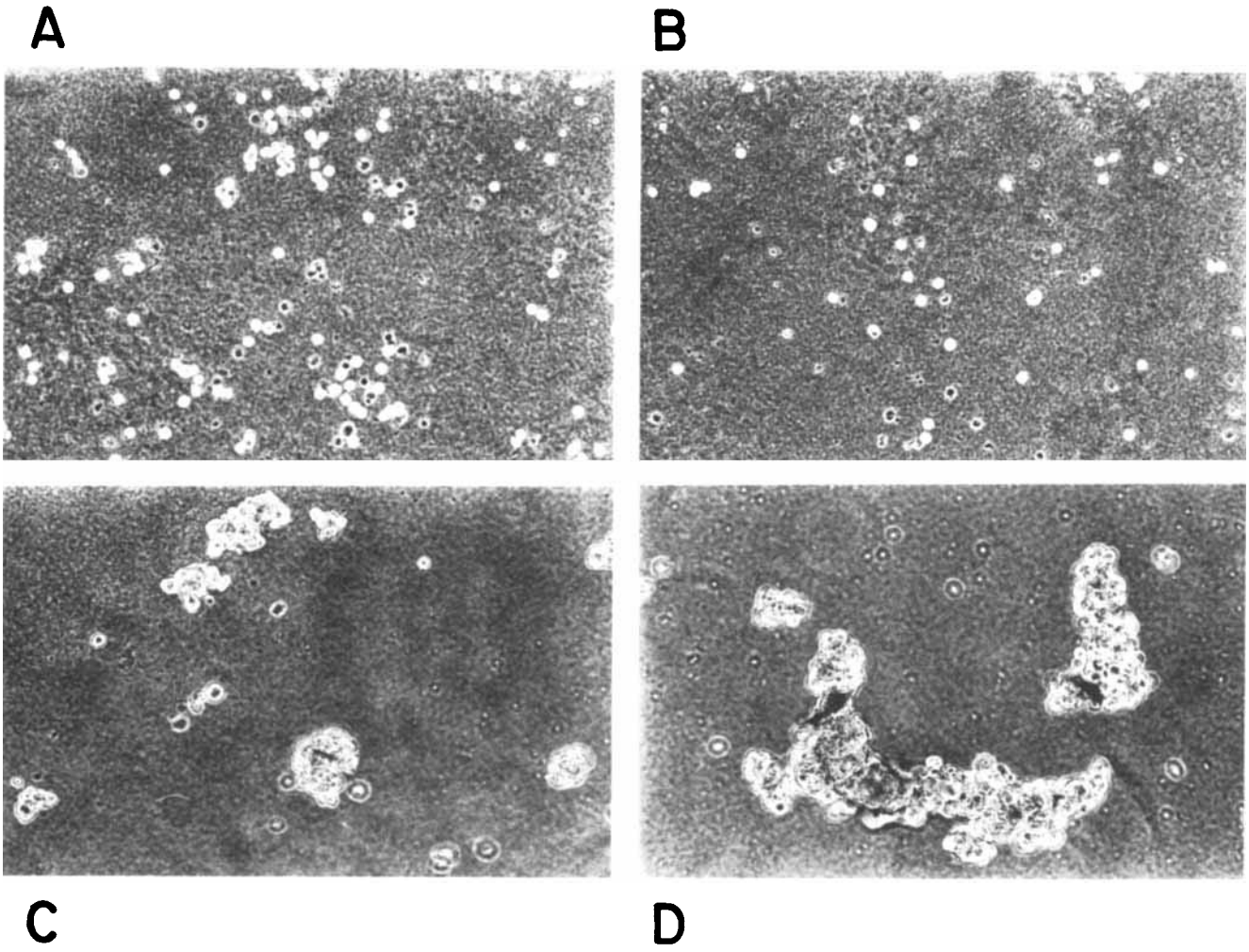

Fig. 4. Aggregates formed after 24 hr in an agar static system (cells were allowed to aggregate undisturbed in liquid growth medium above an agar base layer). Areas of the dish representative of the general behavior were photographed. Normal human fibroblasts (A) and DUP-A cultures (B) were unable to form aggregates, while DUP-N cultures (C) formed several aggregates, whose size was however smaller than that of KHOS/NP cells (D).

were unable to form colonies in soft agar, while KHOS/NP (3%), DUP-N-SV40 (0.8%), and DUP-N cultures (although at a low efficiency of 0.04%) did form tridimensional colonies in agar medium.

Colony formation on epithelial sheet

Analysis of Table 1 shows that normal fibroblasts (964-S, NMS_1, and BMS7) are not able to form colonies on the top of human normal contact-inhibited epithelial sheets (Table 1), while KHOS/NP cells exhibited a very high efficiency (70%); this confirms similar previous results, which were obtained on the same type of cells (Smith et al., 1976). DUP-N and DUP-A cells, contrary to normal cells, were able to form colonies on contact inhibited monolayer of epithelial cells, their efficiency (1%) was, however, lower than that of sarcoma cells. DUP-N-SV40 cultures exhibited a cloning efficiency (15%) that was signficantly higher than that expressed by the untransformed counterpart.

TABLE 2. Immunofluorescent tests

Cell line	964-S	DUP-N	DUP-A
Pericellular fibronectin	++	++	++
Vimentin	+	+	+
Desmin	–	–	–
Actin cables	+	+	+

Growth in low serum concentration

Fifteen days after subcultivation, normal embryonic cells had performed one PD in the presence of low FCS, while in normal adult NMS_1 and BMS7 cells there was no growth at all. In these culture conditions sarcoma cells and DUP-N-SV40 cells performed three and four PDs, respectively, and DUP-N and DUP-A cultures performed two PDs (Table 1), showing a decreased serum requirement in comparison with normal human fibroblasts.

Instability of postconfluent saturation density

Identical confluent cultures were trypsinized, counted, and subsequently passaged at 1:2 and 1:1 split ratios, respectively. After 1:1 splits, we considered positive lines that were able to immediately increase and to grow repeatedly to final densities at least 40% higher than those of identical cultures subcultivated at 1:2 split ratio (Azzarone and Macieira-Coelho, 1982; Azzarone et al., 1981a).

As summarized in Table 1, normal embryonic (964-S) and adult (NMS_1) fibroblasts were not able to increase their final densities after 1:1 splits, while DUP-N (66% increase) and DUP-A (59% increase) were positive for this parameter.

Detection and identification of PA

Human normal resting (964-S, NMS_1, and BMS7) cells and DUP-A cells were found to be negative for the production of PA (Table 1), while DUP-N resting cultures and KHOS/NP cells expressed a very high fibrinolytic activity (1.2 and 1.32 U $\times$ 10^6 cells/hr, respectively), as previously described in several types of transformed fibroblasts (Unkeless et al., 1973). Transformation by SV40 of DUP-N cells caused a sharp increase in the production of PA (5.2 U $\times$ 10^6 cell/hr). The electrophoretic analysis showed that the molecular form of PA produced by DUP-N, KHOS/NP, and DUP-N-SV40 cells was the urokinase like type (MW 60,000).

Immunofluorescent assays

Analysis of Table 2 shows that DUP-N and DUP-A cells, similarly to normal embryonic fibroblasts, exhibit a well-developed pericellular fibronectin network. No difference could be observed in the distribution and organization of actin cables, and the three cell lines expressed the same type of intermediate-sized filaments (vimentin positive, desmin negative).

DISCUSSION

Our results show that cultured cells from a Dupuytren's nodule (DUP-N) and from the apparently normal surrounding palmar aponeurosis (DUP-A) display in vitro characteristics that are intermediate between those expressed by normal human adult (NMS_1 and BMS7) or embryonic (964-S) fibroblasts on one hand and by sarcomatous (KHOS/NP) or virus-transformed (DUP-N-SV40) cells on the other hand. Unfortunately, we have not yet been able for practical reasons to use, as controls, fibroblasts cultured from an age- and sex-matched normal human palmar aponeurosis. Nevertheless, the results of our tests using NMS_1 and in the age- and sex-matched BMS7 fibroblasts correspond to normal values for fibroblasts reported in the literature; moreover, with a single exception (Kopelovich, 1982), there is presently no evidence that normal adult fibroblasts from different locations behave differently in culture as far as the tests used here are concerned (Pfeffer et al., 1976; Smith et al., 1976; Steuer et al., 1977; Kopelovich, 1977; Lanotte et al., 1981).

As with the case of normal cells, DUP-N display contact inhibition of cell division at the plateau phase, limited life span, maximal levels of FCR, high interferon sensitivity, presence of well-developed networks of pericellular fibronectin and cytoplasmic actin cables.

On the other hand, DUP-N cell, similarly to neoplastic cells: (1) form colonies in soft agar, on the top of human contact-inhibited epithelial sheets, (2) aggregate spontaneously in suspension, and (3) grow in the presence of reduced amounts of FCS. It must be noted, however, that generally these "tumoral" features are expressed always in a lower degree than that exhibited by KHOS/NP or DUP-N-SV40 cells. The ability of human embryonic fibroblast to perform, contrary to adult cells, some growth in the presence of low serum concentration confirms previous results (Pfeffer et al., 1976). The chromosome distribution is identical in DUP-N and DUP-A cultures; they show a modal number of 46 chromosomes but also a high percentage of hypodiploid cells, which may represent the symptom of a karyotypic instability. These results are in agreement with a recent description of the presence of important karyologic abnormalities in cell cultures from Dupuytren's nodule and aponeurosis (Sergovich et al., 1983). The production by DUP-N resting cells of high level of the urokinase-like species of plasminogen activator is again typical of transformed cells (Unkeless et al., 1973) and could partially explain the local reactive pathology that characterizes the disease in vivo. It has been reported that plasmin is mitogenic for human fibroblastic cells (Pohjanpelto, 1977): This may contribute, at least in part, to the accumulation of fibroblasts within the nodule.

The ability of DUP-N and DUP-A cultures to increase their final densities after 1:1 splits is a property absent in normal cells (Azzarone and Macieira-Coelho, 1982; Azzarone et al., 1981a; Macieira-Coelho, 1967) that we have previously detected in abnormal skin fibroblast cultures from some cancer patients (Azzarone and Macieira-Coelho, 1982; Azzarone et al., 1981a). We have also shown in this cell model that this particular behavior is due to the selection of a fibroblastic subpopulation less sensitive to cell crowding, which displays increased growth potentials when it is compared to sister cultures routinely subcultured at 1:2 splits (Azzarone and Macieira-Coelho, 1982; Azzarone et al., 1980). This attribute as well as the ability to form colonies on the top of contact-inhibited epithelial cells, could reflect "in vivo" the predisposition of some cells, which display reduced nutritional requirement, to overgrow normal surrounding cells, of similar or different origin. At the same time, these cells, coming reciprocally in contact, could organize tridimensionally, according to their "in vitro" ability to form spontaneous aggregates and to their "in vivo" unusual capacity to establish cell-to-cell contacts (Gabbiani and Majno, 1972) and proliferate in this situation, favoring perhaps the formation of a nodule.

The fact that DUP-N and DUP-A cultures contain intermediate-sized filaments of the vimentin type does not unfortunately allow any speculation about the origin of these cells. It is known that most smooth muscle cells from blood vessels express, as fibroblasts do, vimentin type of intermediate filaments (Gabbiani et al., 1982).

The fact that also DUP-A cultures display a certain degree of deviation from normality suggests that Dupuytren's disease is not strictly focal, and this agrees with previous studies showing the presence of myofibroblasts and of relatively high amounts of type III collagen in the apparently normal aponeurosis of Dupuytren's patients (Bazin et al., 1980) and probably explains the onset of local recurrences. In these patients,

the palmar aponeurosis probably contains many cells that already exhibit decreased nutritional requirement, decreased sensitivity to contact inhibition, and a certain karyotypic instability, but which still lack the ability to organize tridimensionally, to proliferate in this situation, and to produce an enzyme (PA) that could disorganize the structure of the surrounding tissues and act as a mitogen (Pohjanpelto, 1977). The generalization of our results of Dupuytren's disease should be taken cautiously, since they concern only one line derived from a Dupuytren's nodule before and after transformation with SV40 virus and one line derived from the apparently normal aponeurosis of the same patient. Nevertheless, the demonstration that in our conditions Dupuytren's fibroblasts have some of the properties that characterize neoplastic transformation in vitro appears to support the view that this disorder represents a benign tumor of mesenchymal origin (Enzinger et al., 1970).

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LITERATURE CITED

Aron, E. (1968) Le traitement médical de la maladie de Dupuytren par un agent cytostatique (methyl-hydrazine). Presse Med., *76:*1956.

Azzarone, B., and Macieira-Coelho, A. (1982) Heterogeneity of the kinetics of proliferation within human skin fibroblastic cell populations. J. Cell Sci., *57:*177–183.

Azzarone, B., Pedulla, D., and Romanzi, C.A. (1976) Spontaneous transformation of human skin fibroblasts derived from neoplastic patients. Nature, *262:*74–75.

Azzarone, B., Mareel, M., and Macieira-Coelho, A. (1980) Essay of characterization of skin fibroblasts derived from patients with mammary tumours. II. Biological properties of cell cultures from different body sites. In: Cell Movement and Neoplasia. M. de Brabander, M. Mareel and L. De Ridder, eds. Pergamon Press, Oxford, pp. 43–51.

Azzarone, B., Romanzi, C.A., and Macieira-Coelho, A. (1981a) Human skin fibroblasts from patients with mammary tumours: Differences in growth properties. Cancer Detect. Prevent., *4:*249–259.

Azzarone, B., Brouty-Boyé, D., and Macieira-Coelho, A. (1981b) Relationship between fibrin clot retraction and tumorigenesis in C3H/10T1/2 cells. Int. J. Cancer, *28:*799–803.

Azzarone, B., Curatolo, L., Carloni, G., Donati, M.B., Morasca, L., and Macieira-Coelho, A. (1981c) Fibrin clot retraction in normal and transformed avian fibroblasts. J. Natl. Cancer Inst., *67:*89–94.

Bazin, S., Le Lous, M., Duance, V.C., Sims, T.J., Bailey, A.J., Gabbiani, G., d'Andiran, G., Pizzolato, G., Browski, A., Nicoletis, C., and Delaunay, A. (1980) Biochemistry and histology of the connective tissue of Dupuytren's disease lesions. Eur. J. Clin. Invest., *10:*9–16.

Brouty-Boyé, D., Gresser, I., and Baldwin, C. (1979) Decreased sensitivity to interferon associated with in vitro transformation of X-ray-transformed C3H/10T1/2 cells. Int. J. Cancer, *24:*261–265.

Dolfini, E., Azzarone, B., Pedulla, D., Ottaviano, E., De Gaetano, G., Donati, M.B., and Morasca, L. (1976) Characterization of human fibroblasts from cancer patients: Loss of fibrin clot retractile activity after "in vitro" spontaneous transformation. Eur. J. Cancer, *12:*823–825.

Dupuytren, G. (1831–1832) De la réaction des doigts par suite d'une affection de l'aponnévrose palmaire, description de la maladie, opération chirurgicale qui convient dans des cas. J. Univ. Med. Chir. Paris, *5:*352–355.

Enzinger, F.M., Lattes, R., Torloni, H. (1970) Types Histologiques des Tumeurs des Tissus Mous. Classification histologique internationale des tumeurs No 3, Organization mondiale de la Santé, Genève, p. 28.

Failly-Crépin, C., Fromon, S., and Crepin, M. (1981) Dexamethasone effect on the growth rate, the plasminogen activator, and MmTV RNA dependent DNA polymerase in a mouse mammary tumor cell line. Biochem. Biophys. Res. Commun., *102:*22–29.

Foulds, L. (1958) The natural history of cancer. J. Chronic Dis., *8:*2–37.

Gabbiani, G., and Majno, G. (1972) Dupuytren's contracture: Fibroblast contraction? An ultrastructural study. Am. J. Pathol., *66:*131–146.

Gabbiani, G., Chaponnier, C., Zumbe, A., and Vassalli, P. (1977) Actin and tubulin co-cap with surface immunoglobulins in mouse B lymphocytes. Nature, *269:*697–698.

Gabbiani, G., Rungger-Brändle, E., De Chastonay, C., and Franke, W.W. (1982) Vimentin-containing smooth muscle cells in aortic intimal thickening after endothelial injury. Lab. Invest., *47:*265–269.

Granelli-Piperno, A., Vassalli, J.D., and Reich, E. (1977) Secretion of plasminogen activator by human polymorphonuclear leukocytes. Modulation by glucocorticoids and other factors. J. Exp. Med., *146:*1683–1706.

Gresser, I., Fontaine-Brouty-Boyé, D., Bourall, C., and Thomas, M.T. (1969) A comparison of the efficacy of endogenous, exogenous, and combined endogenous-exogenous interferon in the treatment of mice infected with encephalomyocarditis virus. Proc. Soc. Exp. Biol., *130:*236–242.

Hayflick, L., and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res., *25:*585–621.

Kopelovich, L. (1977) Phenotypic markers in human skin fibroblasts as possible diagnostic indices of hereditary adenomatosis of the colon and rectum. Cancer, *40:*2534–2541.

Kopelovich, L. (1982) Are all human normal diploid strains alike? Relevance to carcinogenic mechanisms in vitro. Exp. Cell Biol., *50:*266–270.

Lanotte, M., Allen, T.D., and Dexter, T.M. (1981) Histochemical and ultrastructural characterization of a cell line from human bone marrow stroma. J. Cell Sci., *50:*281–297.

Larsen, R.D. (1966) Dupuytren's contracture. In: Hand Surgery. J.E. Flyn, ed. The William & Wilkins Co., Baltimore, pp. 922–952.

Luck, J.V. (1959) Dupuytren's contracture: A new concept of the pathogenesis correlated with surgical management. J. Bone Joint Surg., *41-A:*635–664.

Macieira-Coelho, A. (1967) Influence of cell density on growth inhibition of human fibroblasts in vitro. Proc. Soc. Exp. Biol. Med., *125:*548–552.

Macieira-Coelho, A., Ponten, J., and Philipson, L. (1966) The division cycle and RNA-synthesis in diploid human cells at different passage levels in vitro. Exp. Cell Res., *42:*673–684.

McAllister, R.M., Reed, G., and Huebner, R.J. (1967) Colonial growth in agar of cells derived from adenovirus-induced hamster tumors. J. Natl. Cancer Inst., *39:*43–53.

Melero, J.A., Stitt, D.T., Mangel, W.G., and Carroll, R.B. (1979) Identification of new polypeptide species (48–55 K) immunoprecipitated by antiserum to purified large T antigen and present in SV40 infected and transformed cells. Virology, *93:*466–480.

Owens, R.B., Smith, H.S., Nelson-Rees, W.A., and Springer, E.L. (1976) Epithelial cells cultures from normal and cancerous human tissues. J. Natl. Cancer Inst., *56:*843–848.

Pfeffer, L., Lipkin, M., Stutman, O., and Kopelovich, L. (1976) Growth abnormalities of cultured human skin fibroblasts derived from individuals with hereditary adenomatosis of the colon and rectum. J. Cell. Physiol., *89:*29–38.

Pohjanpelto, P. (1977) Proteases stimulate proliferation of human fibroblasts. J. Cell. Physiol., *91:*387–392.

Rhim, J.S., Cho., H.Y., and Huebner, R.J. (1975) Non-producer human cells induced by murine sarcoma virus. Int. J. Cancer, *15:*23–29.

Rothfels, K.H., and Siminovitch, L. (1958) An air-drying technique for flattening chromosomes in mammalian cells grown in vitro. Stain Technol., *33:*73–77.

Rungger-Brändle, E., and Gabbiani, G. (1983) The role of cytoskeletal and cytocontractile elements in pathologic processes. Am. J. Pathol., *110:*359–392.

Schneider, E.L., Stanbridge, E.J., and Epstein, C.J. (1974) Incorporation of ^{3}H-uridine and ^{3}H-uracil into RNA. A simple technique for the detection of mycoplasma contamination of cultured cells. Exp. Cell Res., *84:*311–318.

Sergovich, F.R., Botz, J.S., and McFarlane, R.M. (1983) Nonrandom cytogenetic abnormalities in Dupuytren's disease. N. Engl. J. Med., *308:*162–163.

Skoog, T. (1948) Dupuytren's contraction: With special reference to aetiology and improved surgical treatment: Its occurrence in epileptics. Note on knuckle-pads. Acta Chir. Scand., 96 [Suppl.], *139:*1–190.

Smith, H.S., Owens, R.B., Hiller, A.J., Nelson-Rees, W.A., and Johnston, J.O. (1976) The biology of human cells in tissue culture. I.

Characterization of cells derived from osteogenic sarcomas. Int. J. Cancer, *17*:219–234.

Steuer, A.F., Rhim, J.S., Hentosh, P.M., and Ting, R.C. (1977) Survival of human cells in the aggregate form: Potential index of in vitro cell transformation. J. Natl. Cancer Inst., *58*:917–921.

Unkeless, J.C., Tobia, A., Ossowski, L., Quigley, J.P., Rifkin, D.B., and Reich, E. (1973) An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. I. Chick embryo fibroblast cultures transformed by avian RNA tumor viruses. J. Exp. Med., *137*:85–111.

Vincent, R.A. Jr., and Huang, P.C. (1976) The proportion of cells labeled with tritiated thymidine as a function of population doubling level in cultures of fetal, adult, mutant, and tumor origin. Exp. Cell Res., *102*:1–42.

Zardi, L., Carnemolla, B., Siri, A., Santi, L., and Accolla, R.S. (1980) Somatic cell hybrids producing antibodies specific to human fibronectin. Int. J. Cancer, *25*:325–329.