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Intercellular contacts in tumours of the vascular smooth muscle cells in man

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Summary. Electron microscopy has been used to study the cell to cell and cell to stroma contacts between smooth muscle cells (SMC) in normal vessels, angiomyolipoma and well and poorly differentiated vascular leiomyosarcoma. Micrographs were examined with a semiautomatic image analysis system. The length of the cell borders was calculated and the type and number of contacts per 100 μm cell perimeter and per 100 cells were determined. In all cases there was a predominance of simple appositions. Intermediate junctions, nexus junctions, interdigitations, intermediate contacts and junctional interdigitations were less frequently observed. In general, as the SMC become malignant and less differentiated the number of cell to stroma attachments decreases markedly and the intercellular contacts increase slightly.

Key words: Cell contacts – Neoplastic vascular smooth muscle – Human tumours

Introduction

Intercellular adhesion in normal tissues and in tumours is a multifactorial process that is mediated by cell junctions, plasma membrane components, microexudates and divalent ions (Curtis 1973; Staehelin 1974; Garrod and Nicol 1981). It has been suggested that decreased intercellular adhesion is a general property of neoplasm and such decrease in intercellular adhesion may explain certain patterns of tumour growth. It may also be a prerequisite for stromal invasion (Weinstein et al. 1974; Roos 1984).

Cell to cell contacts not only function as sites of mechanical attachment but also serve for intercellular communication (Martinez-Palomo 1971), properties often altered in malignant tumours (Kanno 1985). Although many reports have described the intercellular contacts in carcinoma (Weinstein et al. 1976), there are few studies on tumours of the vascular smooth muscle cells (SMC) and they are without morphological detail (Wang et al. 1974). We therefore decided to investigate the cell to cell and cell to stroma contacts in neoplastic vascular SMC in human tissues. The tumours studied included a well differentiated vascular leiomyosarcoma, a poorly differentiated vascular leiomyosarcoma and the smooth muscle component of an angiomyolipoma.

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Materials and methods

Specimens for this study were obtained from a well differentiated vascular leiomyosarcoma of the inferior vena cava (57 year old woman), a poorly differentiated vascular leiomyosarcoma of the saphenous vein (51 year old woman), the smooth muscle component of an angiomyolipoma of the kidney (27 year old woman) and, as a reference for normal values for the cell contacts in normal vascular smooth muscle, the vascular component of normal kidney biopsies (38 year old woman, 26 year old man).

Although the cell contacts have been studied with different techniques (Henderson 1975), with the material obtained from these rare tumours only transmission electron microscopy was possible. The specimens were fixed with 2.5% glutaraldehyde, in 0.13 M phosphate buffer (pH 7.4) for 1.5 h. The samples were post fixed for 1.5 h in 1% osmium tetroxide, then dehydrated with graded series of ethanol and embedded in araldite. Thin sections were cut in LKB ultramicrotome, picked up on copper grids and stained with uranyl acetate and lead citrate. Sections were examined in a Hitachi H500 electron microscope. Pictures were taken randomly at a magnification of 12000 and each intercellular contact was photographed at higher power for identification and measurement (Alroy et al. 1981). The micrographs were mounted beneath a television camara, connected to a semiautomatic image analysis computer and cell borders were traced with a "mouse". The length of each border was calculated and the type and number of cell contacts per 100 μm cell perimeter (Table 1) and per 100 cells (Table 2) were

Table 1. Contact density per 100 μm of smooth muscle cell perimeter

	Simple appositions	Interdigitations	Intermediate junctions	Nexus junctions	Intermediate contacts	Junctional interdigitations
NV	1.60	0.11	0.19	*	*	*
AGM	0.77	0.03	0.52	*	*	*
WDVL	0.49	0.02	0.20	*	0.18	*
PDVL	1.46	0	1.38	0.08	*	0.04

NV: Normal vessel; AGM: Smooth muscle component of angiomyolipoma; WDVL: Well differentiated vascular leiomyosarcoma; PDVL: Poorly differentiated vascular leiomyosarcoma

* Cell to cell contacts of this type were not identified

Table 2. Contact density per 100 smooth muscle cell profiles

	Simple appositions	Interdigitations	Intermediate junctions	Nexus junctions	Intermediate contacts	Junctional interdigitations
NV	40.77	2.91	4.85	*	*	*
AGM	21.92	0.87	14.91	*	*	*
WDVL	16.96	0.89	7.14	*	6.25	*
PDVL	58.06	*	54.83	3.22	*	1.61

NV: Normal vessels; AGM: Smooth muscle component of angiomyoma; WDVL: Well differentiated vascular leiomyosarcoma; PDVL: Poorly differentiated vascular leiomyosarcoma

* Cell to cell contacts of this type were not identified

determined. Cell to stroma contacts were identified by the presence of electron dense areas (dense bands) on the cytoplasmic side of the plasma membrane in association with basal lamina (Gabella 1984).

Results

In normal SMC there are clusters of mitochondria, rough endoplasmic reticulum and free ribosoms which occupy the zone immediately adjacent to the nucleus. The remainder of the sarcomplasm contains myofilaments associated with dense bodies. The plasma membrane shows on the cytoplasmic side, dense bands alternating with caveolae. Overlying the surface of the cells is a basal lamina which separates individual cell, however, limited areas exist between them in which this substance is lacking and cell to cell contacts are observed.

In human kidney arterioles, the most common type of contact between SMC is found to be a simple apposition with a length of 0.10–1.43 μm and an intercellular space of 10–17 nm (Fig. 1). The intermediate junctions (Fig. 2) measure up to 0.20 μm in length with a membrane separation of 13–15 nm, the electron dense material in these junctions extends into the cytoplasm for up to 0.02 μm. Some SMC show interdigitations (Fig. 3).

The smooth muscle component of the angiomyolipoma shows thick-walled vessels and irregular arranged bundles of SMC in perivascular fashion. The SMC have significant amount of cytoplasmic glycogen and a large number of mitochondria with partial disappearance of myofilaments. Under the plasma membrane the dense plaques are conspicuous and numerous. The cells are surrounded by a basal lamina which shows areas of thickening at the site of apposition of a dense band (Fig. 4).

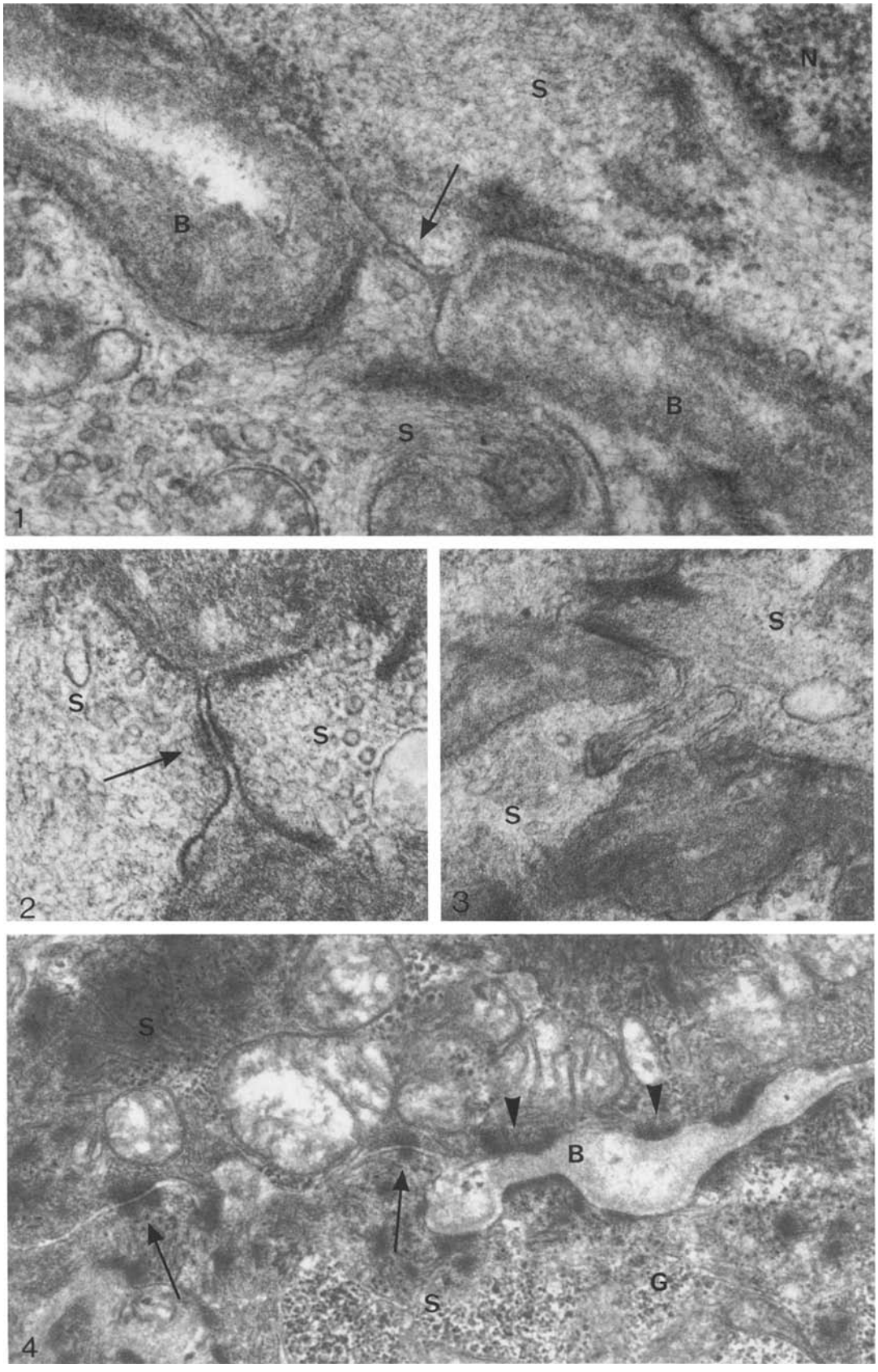
The types of contact identified between SMC are shown in Tables 1 and 2. Simple appositions are the predominant form of contact between cells.

Fig. 1. Simple apposition (*arrow*) between smooth muscle cells (*S*) in normal vessels. There is no membrane or cytoplasmic modification. *B* = Basal lamina; *N* = Nucleus. × 72000

Fig. 2. Intermediate junction (*arrow*) joining smooth muscle cells (*S*) in normal vessels. Note the electron dense material under the plasma membrane. × 66000

Fig. 3. Intercellular contacts mediated by interdigitations. *S* = Smooth muscle. × 37200

Fig. 4. Two smooth muscle cells (*S*) from angiomyolipoma with intermediate junctions (*arrow*). The dense bands are conspicuous (*arrowhead*). *B* = Basal lamina; *G* = Glycogen granules. × 33000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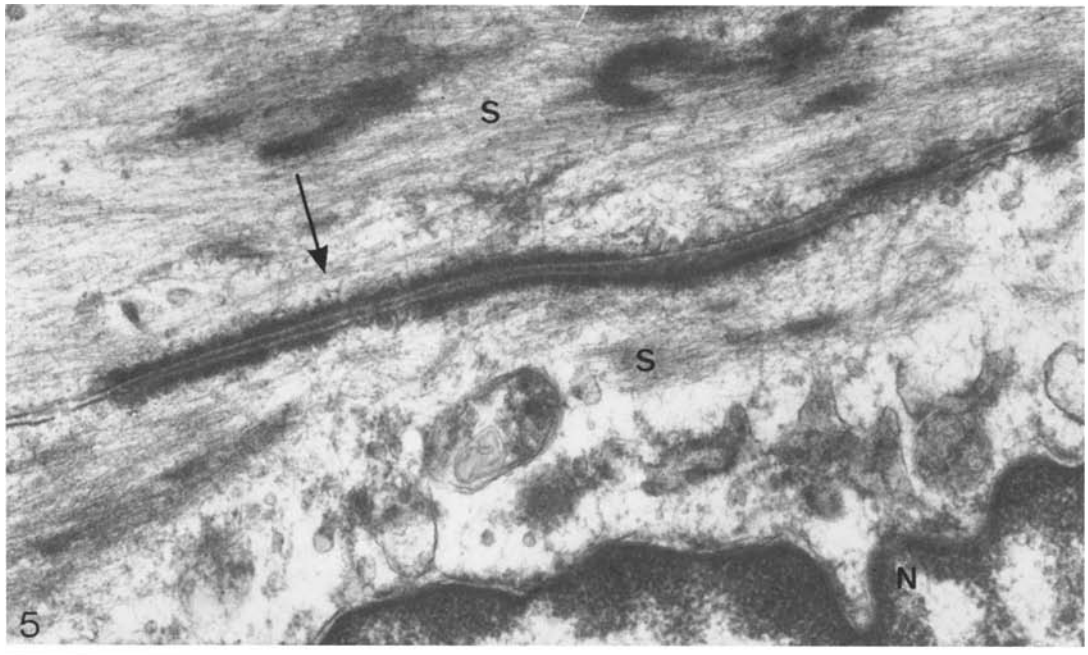
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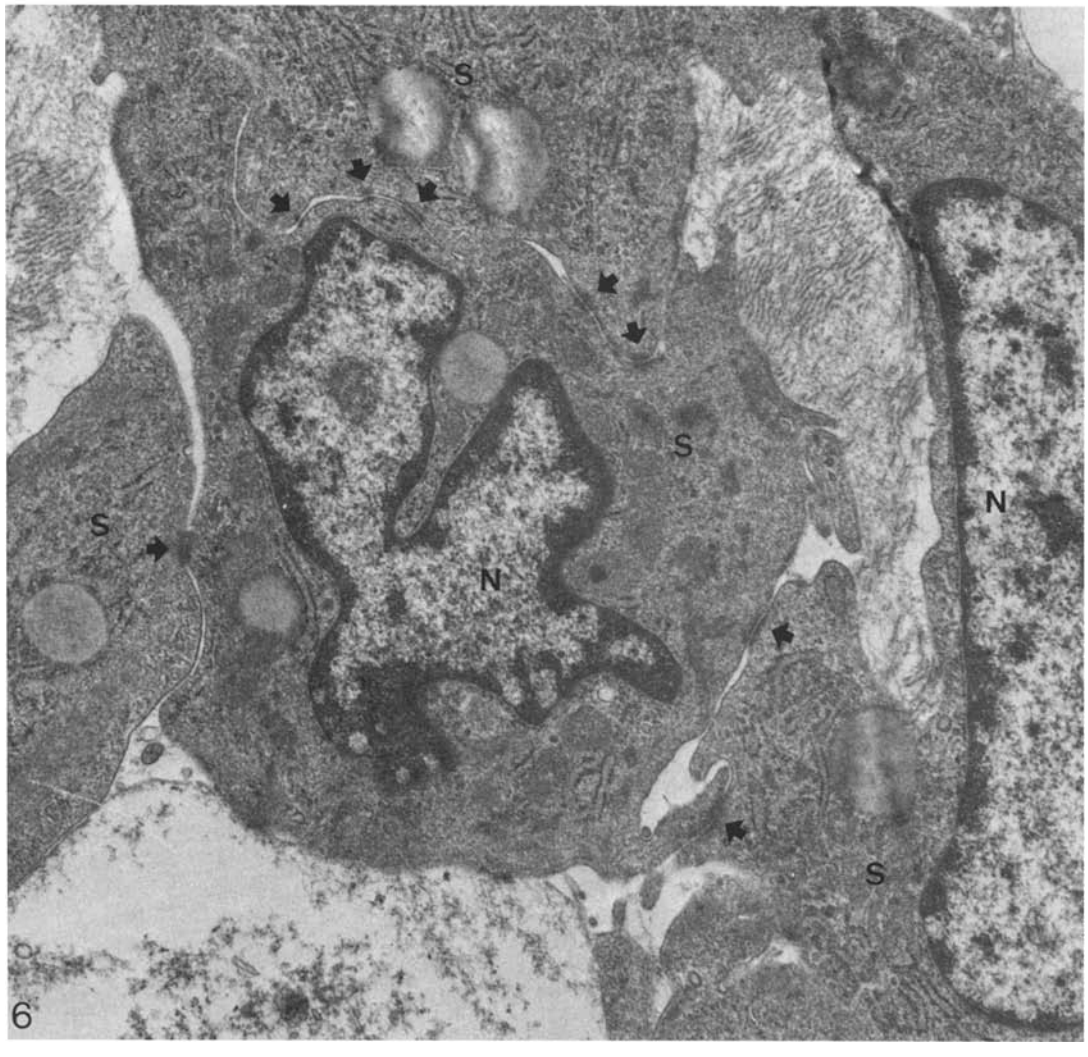
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The area of apposition extends for 0.08–7.50 μm with an intercellular space of 16–25 nm. Intermediate junctions measure 0.08–0.59 μm, with an intercellular space of 10–16 nm (Fig. 4). The electron dense material beneath the plasma membrane is up to 0.03–0.08 μm in thickness. A small number of interdigitations were observed.

In the well differentiated vascular leiomyosarcoma the SMC are elongated with abundant myofilaments and dense bodies orientated parallel to the long axis of the cell. A thin basal lamina is seen enclosing the cells. There is a predominance of simple appositions measuring 1–10 μm in length and the plasma membranes at this point are separated by 11–16 nm. The intermediate junctions are 0.08–0.54 μm in length, an intercellular space of 9–14 nm and the electron dense material beneath the plasma membrane is 0.03–0.09 μm.

In the intermediate contacts, the adjacent plasma membranes have a separation of 42–60 nm. In the centre of the intercellular space there is an electron dense material, which often continues with the basal lamina (Fig. 5). The length of these contacts vary from 0.16 to 1.80 μm and the electron dense material under the plasma membrane is 0.04–0.07 μm.

In the poorly differentiated vascular leiomyosarcoma the cells show sparse myofilaments and prominence of rough endoplasmic reticulum, free ribosomes and fat globules. Dense bands are inconspicuous and the basal lamina is practically absent. The tumour cells show numerous projections along the cell surface and the regions of the plasma membrane not involved in cell to cell contact are separated by enlarged extracellular space containing collagen and elastic tissue.

Simple appositions are the most common type of contact identified, they are 0.08–9.16 μm length with a membrane separation of 11–16 nm. The intermediate junctions (Figs. 6, 7a) measure 0.06–0.57 μm in length, have an intercellular space of 7–16 nm and the electron dense material under the plasma membrane is 0.02–0.06 μm thick. The nexus junctions (Fig. 7b) are 0.16–0.29 μm in length. Some junctional interdigitation are observed (Fig. 7c).

Table 3 shows the results of a search for stromal contacts. The poorly differentiated sarcoma shows a small number of cell to stroma contacts while the smooth muscle in normal vessels, angiomyolipoma and well differentiated vascular leiomyosarcoma contain a relatively large number.

Table 3. Density of cell to stroma contacts

	Per 100 μm of cells perimeter	Per 100 cells
NV	39.77	1008.73
AGM	67.19	1906.14
WDVL	35.22	1226.07
PDVL	0.28	11.29

NV: Normal vessels; AGM: Smooth muscle component of angiomyolipoma; WDVL: Well differentiated vascular leiomyosarcoma; PDVL: Poorly differentiated vascular leiomyosarcoma

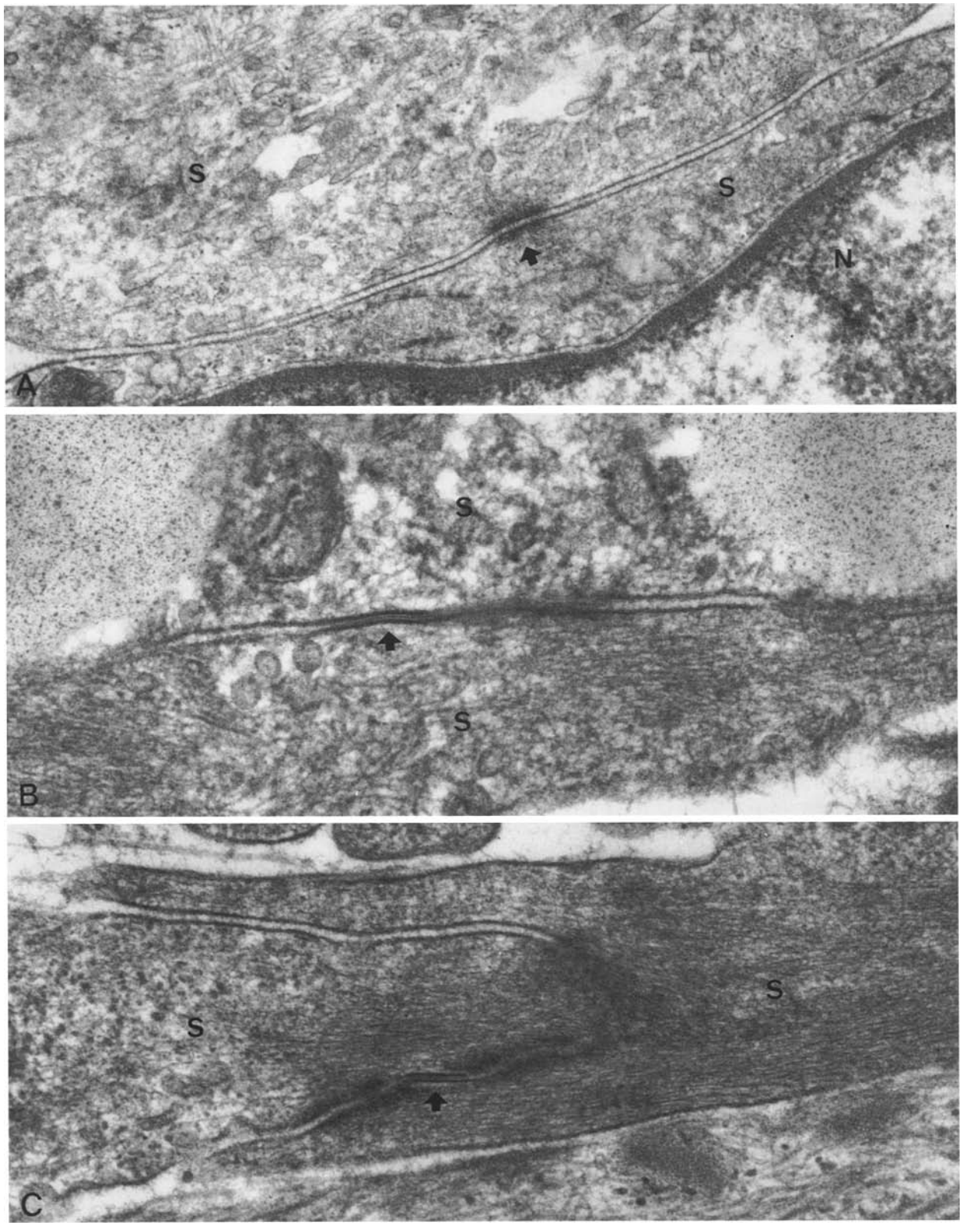
Discussion

In this study, using qualitative and quantitative methods, we have examined cell to cell and cell to stroma contacts in normal and neoplastic vascular SMC in human tissues. The types of contact found include intermediate junctions, nexus junctions, simple appositions, interdigitations, intermediate contacts and junctional interdigitations. The poorly differentiated vascular leiomyosarcoma contains a large number of intercellular contacts but few cell to stroma contacts, the opposite was seen in normal vessels, angiomyolipoma and well differentiated vascular leiomyosarcoma. Cell to stroma contacts in angiomyolipoma are twice as frequent as in normal vessels and, well differentiated vascular leiomyosarcoma contains slightly more cell to stroma contacts than the normal vessels.

In the simple appositions there is no membrane or cytoplasmic specialization although the plasma membranes are closely opposed. These were the most common type of contact observed (Tables 1 and 2). Simple appositions in angiomyolipoma, well and poorly differentiated vascular leiomyosarcoma were up to six times larger than those in normal vessels but the mean intercellular space remains the same. Within the simple appositions there are often intermediate junctions with the same intercellular gap (Fig. 6), although little is known about the possible function of these simple appositions, their frequency and the fact that the

Fig. 5. Cells of a well differentiated vascular leiomyosarcoma (*S*) with an intermediate contact (*arrow*). There is electron dense material in the intercellular space. *N* = Nucleus. × 33000

Fig. 6. Intermediate junctions (*arrows*) of apparent different sizes between cells of a poorly differentiated vascular leiomyosarcoma. *N* = Nucleus. × 18000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intercellular space is usually 10–15 nm are reasons for considering them to be true cell contacts (Henderson 1975).

Intermediate junctions belong to the group of adherens junctions, they hold the cells together mechanically and are associated with actin filaments (Gabella 1984). Although the intermediate junctions have some resemblance to desmosomes, the former lack the dense cytoplasmic plates parallel to the inner membrane leaflets seen in desmosomes. Furthermore, desmosomes are linked to intermediate filaments (Staehelin 1978; Gabella 1984). The intermediate junctions in angiomyolipoma, well and poorly differentiated vascular leiomyosarcoma reached up to twice the size of those in normal vessels, but the intercellular space remains the same. The electron dense material under the plasma membrane is three or four times wider in angiomyolipoma, well and poorly differentiated vascular leiomyosarcoma than in normal vessels.

Intermediate contacts were observed only in the well differentiated vascular leiomyosarcoma. They should be distinguished from intermediate junctions in which the intercellular space is 9–16 nm. Although intermediate contacts have been identified in smooth muscle of gastrointestinal tract, they have not been observed between normal vascular SMC in humans (Henderson 1975).

Interdigitations are identified in all specimens studied. They provide a wider area of cell to cell contact and may show junctions, especially in poorly differentiated vascular leiomyosarcoma (Fig. 7c) where junctional interdigitation is perhaps a more appropiate description. Nexus junctions provide areas of intercellular communication (Bennett and Goodenough 1978; Hooper and Subak-Sharp 1981) and we idenfied them in significant numbers in poorly differentiated vascular leiomyosarcoma.

There are few reports in the literature concerning intercellular contacts in tumours of mesenchymal origin (Clark 1970; Martinez-Palomo 1971) and those associated with vascular smooth muscle tumours in humans are without morphological detail (Wang et al. 1974). In contrast with our results in these vascular lesions, studies in carcinomas indicate that there is a decrease in the number of intercellular contacts (McNutt and Weinstein 1969; Weinstein et al. 1974; Alroy et al. 1981).

Dense bands, consisting of electron dense material attached to the inside of the plasma membrane have been considered to be the major linkage of cells with the stroma (Gabella 1979; Gabella 1984). The interstitial connective tissue matrix and the basal lamina provide mechanical suport and anchor cells in a tissue type-specific structure (Alitalo and Vaheri 1982; Vracko 1982; Liotta et al. 1984). Our results demonstrate loss of dense bands correlating with the loss of differentiation in the vascular leiomyosarcoma.

There are significant differences in the cell to cell and cell to stroma contacts among the different histological tumour types. Alterations in their type and number may play an important role in the invasive behaviour of these neoplastic cells and may provide an aid in evaluating the probable behaviour of mesenchymal neoplasms.

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