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Phenotypic characterization of human keratinocytes in coculture reveals differential effects of fibroblasts from benign fibrous histiocytoma (dermatofibroma) as compared to cells from its malignant form and to normal fibroblasts

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ABSTRACT

Background: Benign and malignant fibrous histiocytoma present with a considerable difference concerning cellular organization in their vicinity.
Objective: Normally appearing epithelium covers the malignant form in contrast to hyperplastic epidermis for benign tumors. It is an open question as to whether the tumor-associated fibroblasts are capable to affect phenotypic features of normal keratinocytes, prompting this comparative analysis.
Methods: Fibroblasts were isolated from benign and malignant fibrous histiocytomas, respectively, and also from normal dermis. The resulting cell populations were thoroughly characterized immunocytochemically using a large panel of antibodies. The three fibroblast preparations were cocultured with normal interfollicular keratinocytes. Their phenotype was characterized for distinct properties including differentiation and proliferation.
Results: Fibroblasts prepared from both tumor types were phenotypically practically identical with normal dermal fibroblasts. Their activities on keratinocytes were different. Cells prepared from benign fibrous histiocytoma were capable to effect strong expression of keratin 19 and production of a galectin-1-rich extracellular matrix. Fibroblasts isolated from malignant fibrous histiocytoma led to a phenotype very similar to that when keratinocytes were cocultured with normal dermal fibroblasts.
Conclusion: Fibroblasts prepared from benign fibrous histiocytoma were biologically active on keratinocytes in a particular manner. Our results on fibroblast activity are suggested to be relevant for morphologic differences observed *in vivo* between normal epidermis and epidermis adjacent to the studied tumor types.

1. Introduction

The fibrohistiocytic tumors of the skin are a heterogeneous group of dermal/subcutaneous mesenchymal neoplasms. "Fibrohistiocytic" refers in this context to a morphologic similarity of the cells with fibroblasts and histiocytes. Indeed, the cells of such tumors show fibroblastic, myofibroblastic and histiocytic (macrophage-like) differentiation, often in the same tumor. The WHO classification (2005) includes benign types of tumors (e.g. fibrous histiocytoma, synonymous: dermatofibroma), tumors of intermediate nature (e.g. plexiform fibrohistiocytic tumor and dermatofibrosarcoma protuberans) and aggresive tumors (e.g. malignant fibrous histiocytoma) [1]. Benign fibrous histiocytoma (BFH) is a common cutaneous soft tissue tumor with a frequency of approximately 3% of the population [2]. Changes of the epidermis in contact with the tumor represent a characteristic diagnostic feature of this type of neoplasia. The epidermal morphology varies from simple acanthosis to pronounced basaloid hyperplasia, very

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similar in appearance to cell clusters in basal cell carcinoma [3–7]. Immunohistochemical analysis of the epidermis overlying the center of the dermatofibroma revealed distinct changes in proliferation and level of differentiation [8]. It is obvious that activation of epidermal keratinocytes may be due to factors originating from tumor and/or stromal cells acting on their environment. The precise origin of fibrohistiocytic tumors has been disputed for decades. Histiocytes, fibroblasts, or cells with intermediate features between fibroblasts and histiocytes and mesenchymal stem cells have all been proposed as origin of the tumor cells. At any rate, an exclusive histiocytic origin is, no longer considered [9].

Emerging insights into the modes of regulating progeny production of epidermal stem cells provide instructive suggestions in this respect [10]. Evidently, adult tissue stem cells acquire proper functionality within a very specialized microenvironment, the so-called niche [11]. Despite recent progress in understanding the complexity of this entity in skin [12], detailed characterization of this type of microenvironment continues to warrant efforts. Because it is generally accepted that mutual mesenchymal–epithelial interactions comprise salient mechanisms of morphogenesis, *in vitro* studies with tumor-derived fibroblasts are an attractive tool toward further progress. Of relevance in this context, cellular parameters of fibroblasts are significantly influenced by their site of localization with ability to maintain these features under physiological conditions [13], and they can be the source of modulatory effects as e.g. demonstrated by expression of distinct types of keratins in cocultured keratinocytes [14]. Following this line of evidence a regulatory role of cancer-associated stromal fibroblasts on the biology of neighboring cells including tumor cells was delineated [15]. Thus, when fibroblasts were prepared from basal or squamous cell carcinomas, they were active to influence phenotypic features of normal human keratinocytes [16,17]. At this stage, it is an open question as to whether stromal fibroblasts from a benign tumor have a similar activity, which may underlie establishment of morphological features of the tumor *in situ*.

In this study, we addressed this issue and answer the question on a possible role of fibroblasts from BFH on normal human keratinocytes using an *in vitro* cocultivation model. As internal standard we have run assays in parallel with cancer-associated stromal fibroblasts (CASF) from MFH. This tumor type is morphologically diverse including the presence of condensed, fibroblast-rich stroma (similar to BFH and basal cell carcinoma) but lacking appearance of strong hyperplasia with no hyperplastic changes in the adjacent epidermis [18,19]. The use of immunohistochemistry can be valuable in the diagnostic workup of any spindle-cell fibrohistiocytic tumors, diagnosis of MFH based on morphology alone not being reliable. There are no markers or combinations of markers that establish the diagnosis of malignant fibrous histiocytoma. The tumor cells of MFH can often show a "vimentin only" immunophenotype with no ability of other immunostains to discern any marked sign of differentiation. The lesional cells of MHF must be negative for cytokeratins and S100 protein; a small extent of expression of actin, indicating myofibroblastic differentiation, is acceptable. Fibrohistiocytic tumors usually contain nontumoral S100 protein-positive Langerhans cells, CD31-positive endothelial cells and macrophages, as well as factor XIIIa-positive dendritic cells. CD68 expression does not support or exclude the diagnosis of MHF in line with the other traditional histiocytic markers (α_1-antitrypsin, α_1-antichymotrypsin, and factor XIII) [20].

We isolated CASF from both types of tumor and cocultured them with normal interfollicular keratinocytes to probe into and to characterize biological effect(s) triggered by the studied fibroblasts. To establish an internal reference value we added experimental series with normal dermal fibroblasts (DF). Based on our previous studies with basal/squamous cell carcinoma [16,17], in which we demonstrated a shift of phenotype of normal keratinocytes cocultured with tumor stromal cells, we focused analysis on keratin 8 (that is present in squamous cell carcinoma and not in normal epidermis and basalioma), on keratin 19 (that is present in bulge epidermal stem cells and in a minimum of 50% of basalioma) and on vimentin. Its coexpression with keratins is indicative of epithelial–mesenchymal interaction. Expression of nucleostemin, binding sites for Gal-1 and Ki 67 can be related not only to proliferation but also to differentiation status of keratinocytes monitored (for details see [16,17]).

2. Material and methods

2.1. Tissue preparation and cell culture

Specimens of BFH and MFH (one specimen of each tumor) were obtained from the Department of Dermatovenereology of the 1st Faculty of Medicine (Charles University, Prague, Czech Republic), and tissue for control, i.e. normal skin, came from the Department of Aesthetic Surgery of the 3rd Faculty of Medicine of the Charles University in Prague, in all cases with written informed consent of the donors. The experiments were approved by local ethical committee and performed strictly according to the Declaration of Helsinki principles. A part of each tumor and of normal skin was fixed with paraformaldehyde, embedded in paraffin and used for routine pathologic characterization after hematoxilin and eosin staining and for processing by immunohistochemistry (panel of keratins, keratin 19, galectin-1; for details, please see below).

Normal DF and keratinocytes were prepared by mild trypsinization overnight and cultured by a modified Rheinwald and Green procedure [21], as described previously [16,17]. Fibroblasts present at the site of the tumor were isolated and cultured according to a routine protocol [22] with modifications given elsewhere [16,17]. Cells with normal fibroblastoid appearance were used from the seventh passage cultured for 53 days in the case of BFH and from the sixth passage cultured for 77 days for MFH, respectively. Their phenotype was repetitively examined by the detection of vimentin, keratins and CD68 (please see below). This procedure ensured to work with fibroblasts. Feeder cells were seeded on cover glass at the low density of 4,000 cells/cm^2 and cultured for 24 h, the suspension of keratinocytes (30,000 cells/cm^2) was then added, cells were then kept in culture in a keratinocyte medium (DMEM + F12, 3:1) at 37 °C and 5% CO_2 [16,17] for 5 days. This experiment was repeated up to five times independently to ascertain reproducibility.

2.2. FACScan analysis of fibroblasts

The cultured fibroblasts prepared from both types of tumor and from normal dermis were analysed after trypsinization using FACSCalibur® equipment (BD Biosciences, Heidelberg, Germany), and data processing followed using the Summit® V3.3. Build 1024 software (DakoCytomation, Fort Collins, CO, USA) [16,17]. Single-cell suspensions in phosphate-buffered saline (PBS) containing 2% fetal calf serum were characterized by probing for presence of the following markers: cluster of differentiation markers CD11b, CD18, CD29, CD44, CD45, CD49a, CD49d, CD63, CD90, CD106, and CD166 (all from Becton Dickinson, Prague, Czech Republic), CD11c, CD14, CD34, CD45, CD68, CD71, CD235a, CD105, HLA DR, DQ, DP and HLA-A, -B, and -C (all from Dako, Brno, Czech Republic), CXCR4, and alkaline phosphatase (R&D Systems, Minneapolis, MN, USA) as well as CD19e and CD49c (Chemicon, Temecula, CA, USA). Isotype immunoglobulins were used as negative controls in all experiments.

2.3. Immunocytochemistry

Samples of normal epidermis, and epidermis surrounding the tumors (BFH and MFH) were paraffinized and routinely stained for presence of a panel of keratins, keratin 19 and galectin-1 after the retrieval of antigen (Antigen Unmasking Solution; Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instruction- for the description of staining procedure, please see below).

Fibroblasts prepared from both types of tumors and keratinocytes kept in coculture, as was also the case with normal DF, were characterized immunocytochemically by multiple labelling at the single-cell level as described in detail elsewhere [16,17]. Fibroblasts were processed to detect presence of the macrophage tandem-repeat-type mannose receptor (Gaubius Laboratory, TNO Prevention and Health, Leiden, The Netherlands), CD14 (MEM 15 antibody; generous gift of Prof. V. Hořejší, Institute of Molecular Genetics of the Academy of Science, vvi., Prague, Czech Republic), CD45 (Sigma–Aldrich, Prague, Czech Republic), CD34, CD68, CD71, vimentin, smooth muscle actin, Ki67 (DAKO Cytomation, Brno, Czech Republic) and nucleostemin (Neuromics, Bloomington, MN, USA). Keratinocytes were characterized by determining presence of a panel of keratins using a rabbit polyclonal antibody (Abcam, Cambridge, UK). Keratin 8 was detected by a mouse monoclonal antibody (DAKOCytomation, Brno, Czech Republic) and keratin 19 by a mouse monoclonal (Sigma–Aldrich, Prague, Czech Republic). Epithelial–mesenchymal transition zone was defined immunocytochemically by the occurrence of coexpression of keratins with vimentin (see above). Nucleostemin and Ki67 were also detected (as mentioned above). As a common marker of tumor stroma the endogenous lectin galectin-1 was visualized using a home-made polyclonal rabbit anti-human galectin-1 antibody, rigorously checked for absence of cross-reactivity with other galectins [23–25], in coculture of keratinocytes with experimental fibroblasts. Western blotting of cell extracts comparing mock-treated and galectin-1-overexpressing transfected cells with strong ectopic expression was performed as further control, running highly sensitive signal visualization by chemiluminescence [26,27].

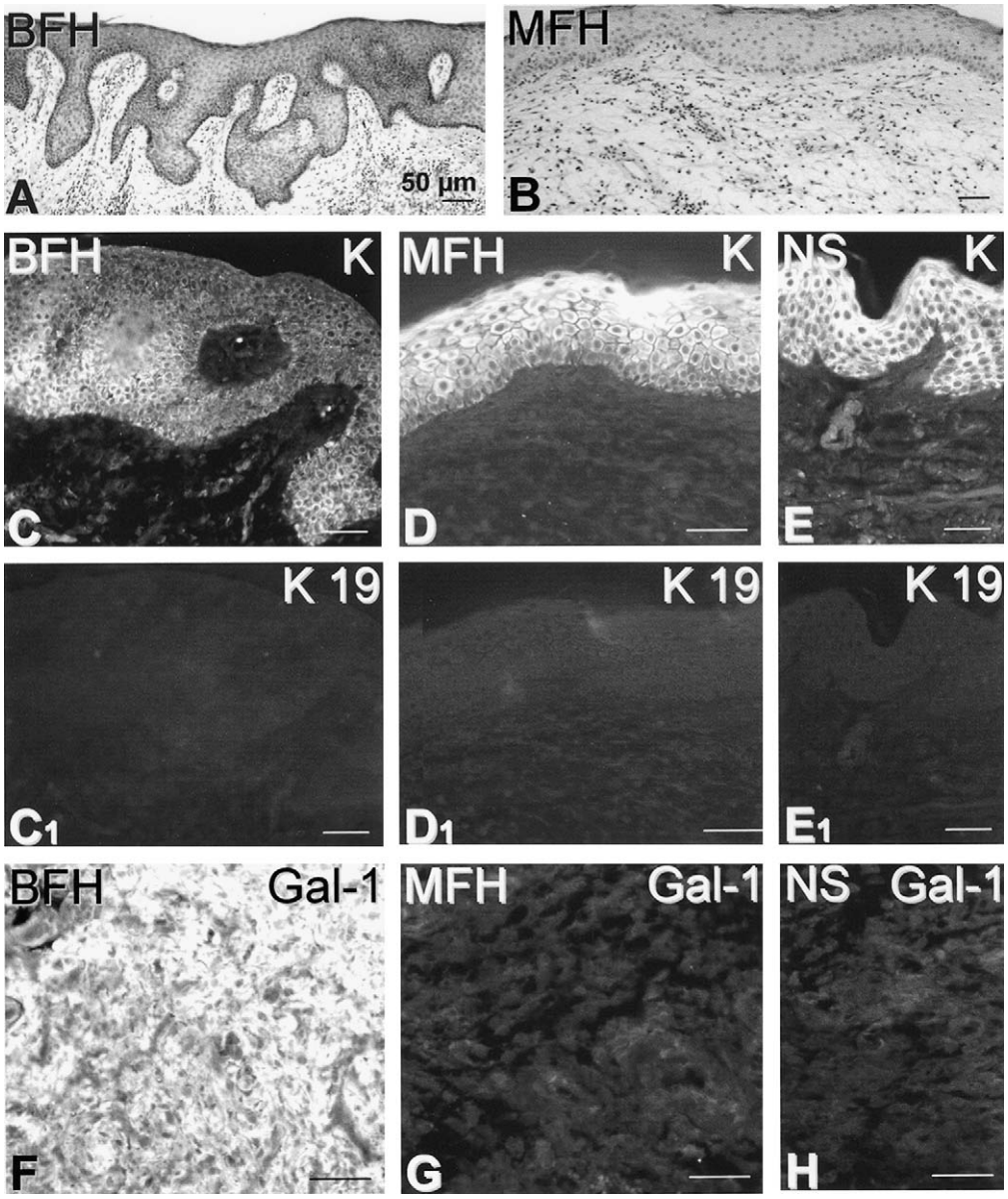

Fig. 1. Benign fibrous histiocytoma (BFH) with extensive hyperplastic epidermis (A) in contrast to malignant fibrous histiocytoma (MFH) covered by epidermis with normal appearance (B). Epidermis above both types of tumors (C and D) resembled epidermis of normal skin (NS), (E) in terms of absence of keratin 19 (C_1–E_1). Stroma of BFH contains a high level of the endogenous lectin galectin-1 (Gal-1) (F) whose extent of expression is comparatively low in MFH (G) and in dermis of normal skin (H). Bar is 50 μm.

Purification and biotinylation, controls for purity and binding activity as well as measuring degree of labelling of this human lectin were described in detail elsewhere [24,28,29]. Fibronectin as extracellular matrix component, a glycoprotein ligand for galectin-1, was also detected in cultured cells with rabbit polyclonal antibody (DAKOCytomation, Brno, Czech Republic). Fixation procedure and dilution of primary antibodies were set according to the recommendation of the corresponding suppliers. FITC-labeled swine anti-mouse serum (AlSeVa, Prague, Czech Republic) was the second-step reagent in the cases of CD14, CD34, CD45, CD68, CD71 and vimentin, FITC-labeled swine anti-rabbit serum (AlSeVa, Prague, Czech Republic) for processing to detect the macrophage tandem-repeat-type mannose receptor, galectin-1 and the panel of keratins, respectively. TRITC-labeled goat anti-mouse serum (Sigma–Aldrich, Prague, Czech Republic) facilitated visualization of signals for vimentin, keratin 8, keratin 19 and smooth muscle actin, TRITC-labeled donkey ant-goat serum (Jackson Laboratories, West Growe, PA, USA) for nucleostemin. Control experiments were performed by replacement of specific antibodies by mono- or polyclonal antibodies with specificity that is irrelevant in the studied cells and tissues (in the case of monoclonals of the same isotype). DNA visualization by DAPI (4′,6′-diamidino-2-phenyindole dilactate; Sigma–Aldrich, Prague, Czech Republic) provided a signal for the cell nucleus. Specimens were then mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA) and inspected using an Eclipse 90i (Nikon, Prague, Czech Republic) fluorescence microscope

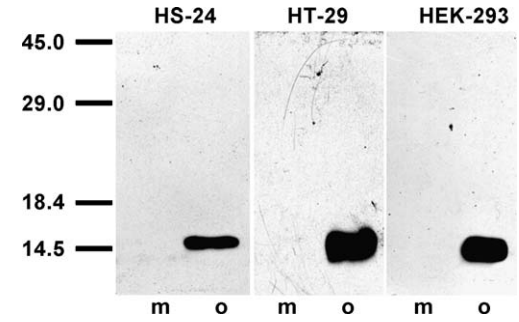

Fig. 2. Western blot analysis of extracts of mock-treated (m) and galectin-1-overexpressing (o) cells (50 μg protein per lane; molecular weight markers designated by mass) to illustrate specificity of the anti-galectin-1 immunoglobulin G preparation. Extracts from human HS-24 non-small cell lung cancer (left), HT-29 colorectal adenocarcinoma (center) and HEK 293 embryonic kidney (right) cells were processed.

equipped with the suited filterblocks, a high-resolution CCD camera (Vosskühler Cool-1300Q; Vosskühler, Osnabrück, Germany) and a computer-assisted image analyzer (LUCIA 5.10; Laboratory Imaging, Prague, Czech Republic). In addition to routine documentation microscopy was also performed for acquiring quantitative data on populations of 500 cells per specimen. The statistical significance was tested using the Student *t*-test. Any difference with a level lower than 0.05 was considered to be statistically significant.

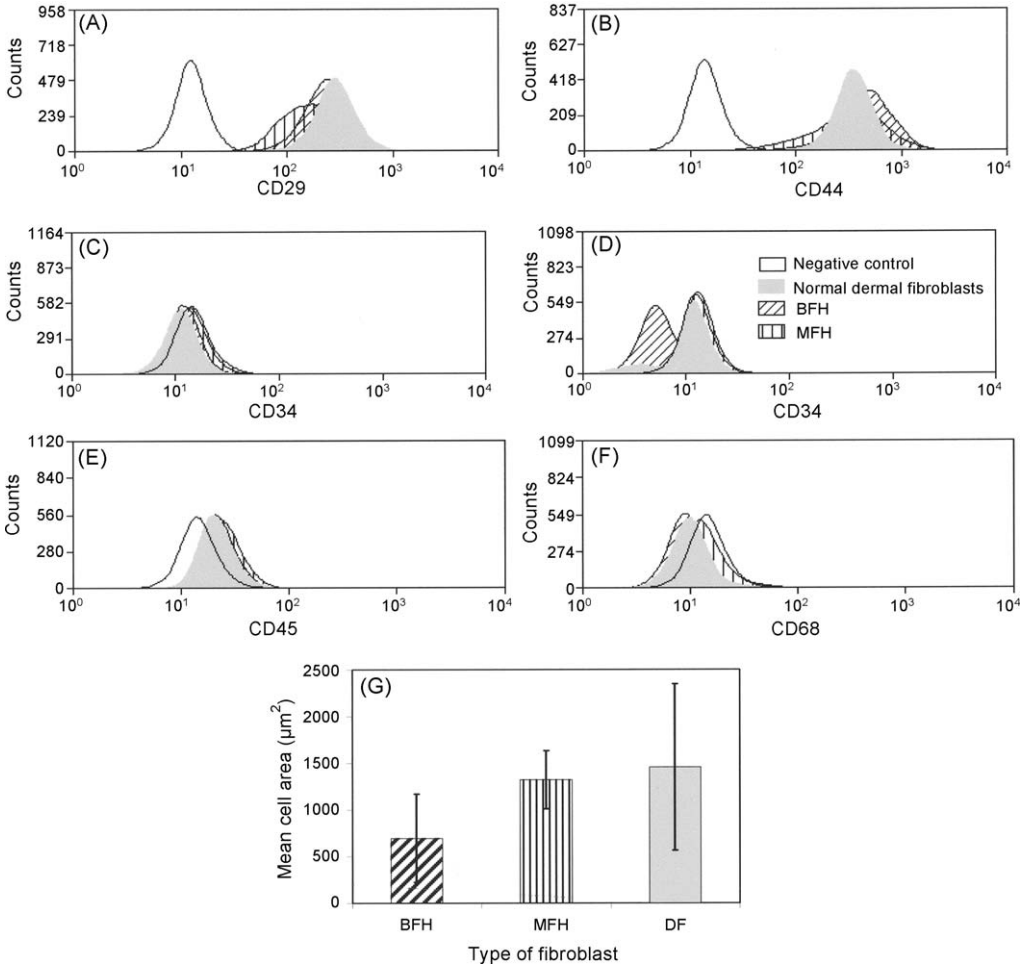

Fig. 3. Representative documentation of intensity of immunodetection of selected markers in normal dermal fibroblasts and fibroblasts prepared from BFH and MFH (A–F). While all three types of cells are positive for CD29 (A) and CD44 (B), they were negative for CD14 (C), CD34 (D), CD45 (E) and CD68 (F). The size of cells when measured after adhesion and spreading was lower in the case of fibroblasts prepared from BFH than that of cells originating from MFH and of normal dermal fibroblasts, the difference not reaching the level of statistical significance (G).

3. Results

3.1. Characterization of epidermis

Epidermis overlying BFH showed the characteristic hyperplasia in contrast to a rather normal appearance of epidermis when monitoring MFH (Fig. 1A and B). Epidermis above both types of tumor expressed keratins, as seen in normal epidermis (positive control of accessibility of antigen for antibody) (Fig. 1C–E). Keratin 19 was not detected in both normal interfollicular epidermis and in the epidermis in contact with both studied tumors (Fig. 1C_1–E_1). In contrast to normal skin and MFH, BFH exhibited an intense signal for presence of galectin-1 in stromal component (Fig. 1F–H). The antibody preparation did not cross-react with other members of the family of human galectins and its specificity was further ascertained by Western blotting with human cell extracts (Fig. 2).

3.2. Characterization of fibroblasts

Fibroblasts prepared from both types of tumor presented a similar phenotype without major deviation from appearance of normal DF (Fig. 3A–F; Fig. 4 A–H; Table 1), with several notable exceptions. They express no markers typical for leukocytes/histiocytes (CD11, CD14, CD18, CD45, CD49, CD63, CD68, CD71, CD90, CD105, CD166, CD235, CXCR4), for hemopoietic precursor and endothelial cells (CD34, CD105, CD106, CD166) and for mesenchymal stem cells (alkaline phosphatase). Also, the expression profile of both HLA-I/II determinants was identical with

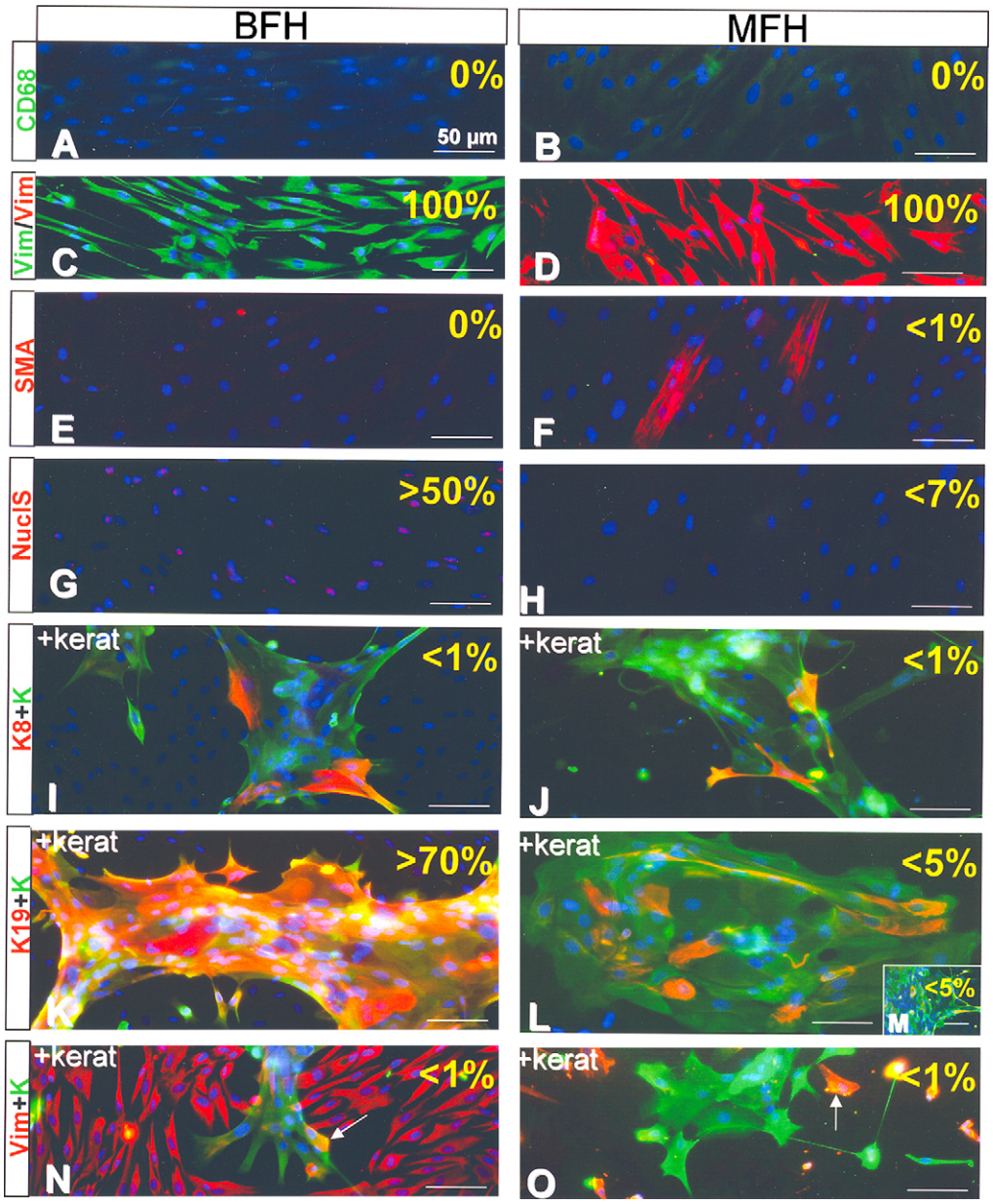

Fig. 4. Fibroblasts prepared from both types of tumor are negative for CD68 (A and B) and highly positive for vimentin (Vim) (C and D). Whereas no smooth muscle actin (SMA)-containing myofibroblasts were present among the fibroblast population prepared from BFH (E), these cells, albeit at very low frequency, were detected in the pool of fibroblasts prepared from MFH (F). A high proportion of nuclei of fibroblasts from BFH is positive for nucleostemin (NuclS) (G), No nucleostemin was present in nucleoli among fibroblasts prepared from MFH (H). Keratin 8 (K8) was detected in a very low number of keratinocytes cocultured with fibroblasts prepared from BFH (I) and MFH (J). Coculture of BFH-derived fibroblasts with keratinocytes led to a strong staining for keratin 19 (K19) in these cells (K). Very low level of presence of K19 was observed in keratinocytes cocultured with fibroblasts from MFH (L) and with normal dermal fibroblasts (M). Coexpression of keratins with vimentin (Vim, arrow) was negligible in keratinocytes cocultured with BFH- (N) and MFH-derived fibroblasts (O). Bar is 50 μm.

Table 1
Phenotypic characterization of studied fibroblast populations by FACScan analysis.

Marker	NDF	BFHF	MFHF
CD11b	–	–	–
CD11c	–	–	–
CD14	–[a]	–[a]	–[a]
CD18	–	–	–
CD29	+	+	+
CD34	–[a]	–[a]	–[a]
CD44	+	+	+
CD45	–[a]	–[a]	–[a]
CD49a	–	–	–
CD49c	–	–	–
CD49d	–	–	–
CD49e	–	–	–
CD63	–	–	–
CD68	–[a]	–[a]	–[a]
CD71	–[a]	–[a]	–[a]
CD90	–	–	–
CD105	–	–	–
CD106	–	–	–
CD166	–	–	–
CD235a	–	–	–
CXCR4	–	–	–
HLA-I	+	+	+
HLA-II	–	–	–
Alkaline phosphatase	–	–	–

NDF: normal dermal fibroblasts BFHF; fibroblasts from benign fibrous histiocytoma MFHF: malignant fibrous histiocytoma
[a] Assessment was ascertained immunocytochemically

normal fibroblasts. CD29 and CD44 positivity indicates that all three types of evaluated cells have the same ability to interact with components of the extracellular matrix. In contrast to fibroblasts prepared from BFH and to normal DF, myofibroblasts were present in the pool of cells prepared from MFH (Fig. 4E and F). Of further note, the number of nucleostemin-positive cells was significantly increased ($p < 0.001$) in fibroblasts originating from BFH (Fig. 4G and H). Overall, these fibroblasts were somewhat smaller than those prepared from MFH and from normal dermis. However, the differences did not reach the level of statistical significance (Fig. 3G, $p = 0.08$). Summarizing the immunophenotyping carried out with the three cell preparations (Table 1), the cells used for further *in vitro* coculture experiments with normal keratinocytes were fibroblasts, with no other elements being present in the population of cultured stromal cells of BFH and MFH.

3.3. Characterization of the keratinocytes cocultured with fibroblasts

First examining morphology, keratinocyte colonies cocultured with fibroblasts prepared from both types of tumor and normal dermis had a rather similar appearance (Fig. 4I–O, Fig. 5A–K). Next, the status of differentiation was monitored based on keratin immunocytochemistry. While only very few keratinocytes cultured on all three types of fibroblasts (MFH, BFH, normal skin) were positive for keratin 8 (Fig. 4I and J), keratinocytes grown in coculture with fibroblasts prepared from BFH significantly ($p < 0.02$) expressed keratin 19, a definitively distinctive feature (Fig. 4K and L). Expression of this type of keratin in keratinocytes cocultured with normal dermal fibroblasts (Fig. 4M) was practically identical with appearance in those cultures where keratinocytes were grown together with fibroblasts prepared from MFH (Fig. 4L). Presence of keratinocytes coexpressing both keratins and vimentin was negligible in coculture with all types of fibroblasts (Fig. 1N and O). This observation excludes a significant extent of epithelial–mesenchymal transition in any of the tested systems. The adhesion/growth-regulatory endogenous lectin galectin-1, known to be expressed in the stroma of various tumors including basal cell carcinoma *in situ* and *in vitro* [25,30], was part of deposits of the extracellular matrix produced by fibroblasts from BFH cocultured with keratinocytes (Fig. 5A–C). These deposits also contained the glycoprotein fibronectin, a ligand of this lectin (not shown). The majority of nuclei of keratinocytes cocultured with all three types of fibroblasts harbored presence of galectin-1-binding sites (Fig. 5D and E). In comparison, the signal was rather strong in nuclei of keratinocytes cocultured with fibroblasts from BFH, a situation also encountered when keratinocytes were cocultured with normal dermal fibroblasts, this result being quantitatively substantiated by measuring the profile of fluorescence intensity (Fig. 5D–G).

Next, the proliferation status of the keratinocytes was determined by monitoring Ki67 presence. Its extent was apparently higher for keratinocytes in coculture with fibroblasts prepared from BFH, this difference yet not passing the threshold for statistical significance ($p = 0.09$) (Fig. 5H and I). A further difference concerned expression of nucleostemin. Interfollicular keratinocytes in coculture with DF revealed no signal for nucleostemin (not shown), practically all cells cocultured with both types of tumor fibroblasts contained nucleostemin-positive nucleoli (Fig. 5J and K). Overall, the characterization of the phenotype of interfollicular keratinocytes cocultured with fibroblasts prepared from BFH revealed an influence akin to the effect on keratinocytes when cocultured with fibroblasts prepared from basal cell carcinoma [16].

4. Discussion

Although the marked hyperplasia of epidermis overlying BFH is well known as morphological feature and used in diagnostic procedures [3–8], the mechanisms underlying this phenomenon are yet to be defined. Rather likely, stromal fibroblasts may play a role in this cascade of processes, because fibroblasts prepared from both basal and squamous cell carcinoma, respectively, are able to significantly influence the phenotype of keratinocytes in coculture [16,17]. Our current experiments were designed to address this issue. As an essential prerequisite due to the heterogeneous nature of tumor stroma in these cases, thorough and extensive characterization of experimental fibroblasts was mandatory [18,19,31]. Fibroblasts prepared from the both types of tumors, i.e. BFH and MFH, were phenotypically identical with normal dermal fibroblasts except for the presence of rare cases of cells exhibiting a signal for smooth muscle actin in cultured cells prepared from MFH. The comparative phenotypic investigation of the cells prepared from both types of tumor indicated that these cells can reliably be considered as fibroblasts. High level of expression of nucleostemin in nuclei of cells prepared from MFH can be related to their enhanced proliferation potential and low differentiaton status [32], and myofibroblasts are frequently present in malignant tumor stroma [33].

When grown in contact to fibroblasts prepared from BFH, keratinocytes were found to strongly express of keratin 19. A similar effect on the keratinocyte population had previously been observed, in coculture with stromal cells prepared from basal cell carcinoma [16]. Keratin 19 is present in epidermal stem cells under physiological conditions [34], and this cytoskeletal protein is also detectable in cells of basal cell carcinoma [35]. In our previous study, we had observed that keratin 19 could also be transiently induced in a fraction of the population of basal interfollicular keratinocytes after a suspension regimen [36]. In contrast, no substantial presence of keratin 8 was seen in keratinocytes under the influence of BFH-derived fibroblasts, a feature common for normal keratinocyte coculture with fibroblasts from squamous cell carcinoma [17]. Because this keratin protein is normally not present in postnatal squamous epithelia and is typical for malignant cells of squamous cell carcinoma of the head and neck

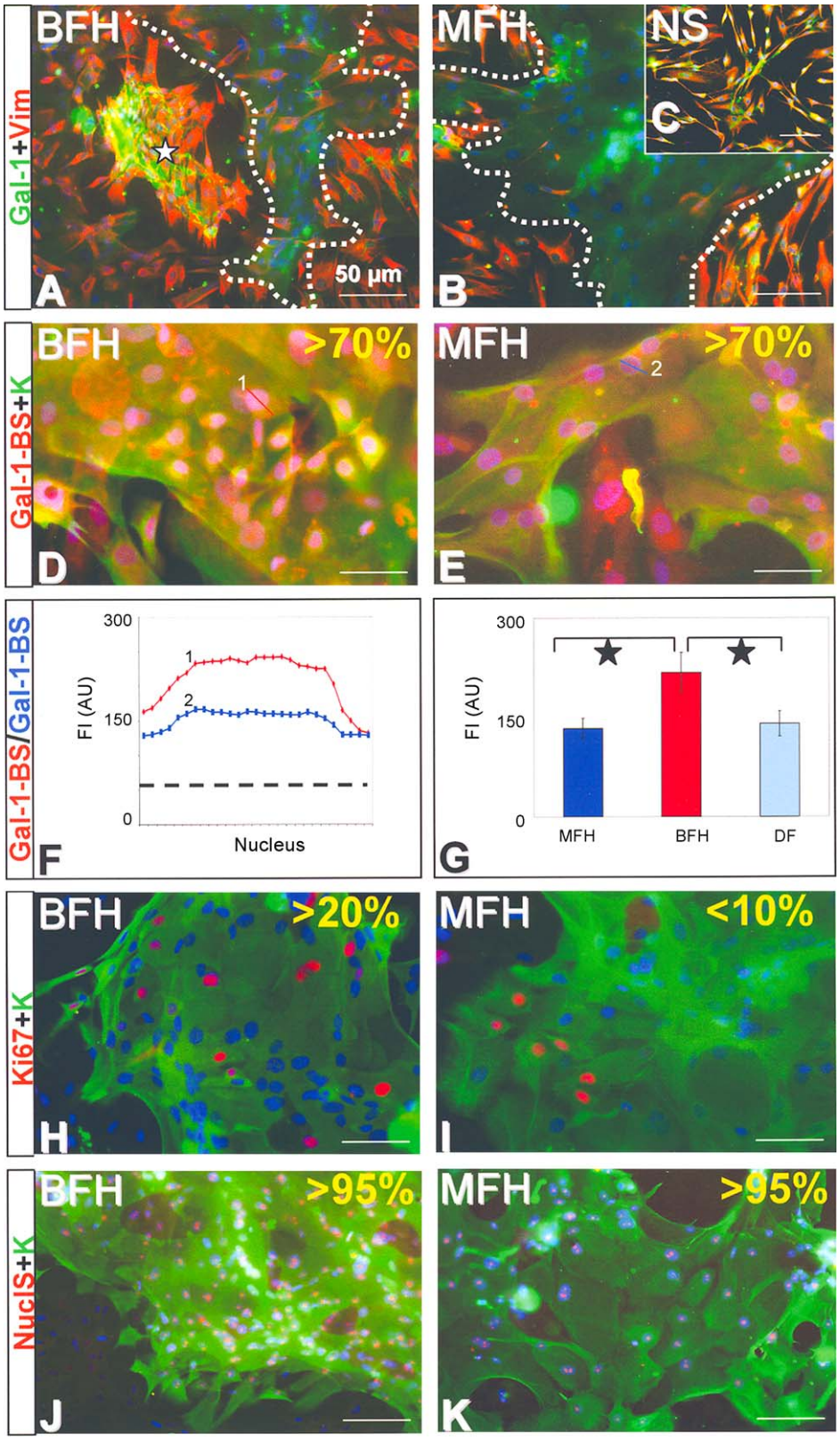

Fig. 5. Fibroblasts prepared from BFH cocultured with interfollicular keratinocytes characteristically produce galectin-1 (Gal-1)-rich extracellular matrices (white star, A) that are not seen in parallel experiments with either cells from MFH (B) or from normal dermis (C). Majority of keratinocyte nuclei were reactive with Gal-1 in both cell populations cocultured with fibroblasts prepared from BFH (C) and MFH (D). Measuring the fluorescence intensity profile of Gal-1 binding, the intensity of reactivity was significantly increased in the cell system starting from BFH and from normal dermal fibroblasts (DF) (at the significance level of $p = 0.01$ in the case of MFH and $p = 0.03$ in the case of DF) (F and G). Representative profiles are marked by numbers 1 and 2 (D and E). Presence of the proliferation marker Ki67 could apparently be observed more frequently (not reaching the $p < 0.05$ threshold) in the system containing BFH (H) than in that from MFH (I). Also, the expression of nucleostemin was not significantly influenced by the origin of cells from BFH and MFH (J and K). The non-paired Student *t*-test was applied to process data statistically, differences with $p < 0.05$ being considered as statistically significant.

of patients with poor prognosis [37], the influence of BFH-associated fibroblasts appears distinct and separate from the effect of fibroblasts originating from squamous cell epithelia. This observation is corroborated by a practically absent coexpression of keratins with vimentin, a protein that defines the epithelial–mesenchymal transition [17,38,39]. The difference between absence of keratin 19 in epidermis positioned over the tumor in BFH *in situ* and the positive signal from keratinocytes cocultured with fibroblasts prepared from this tumor can be due to differences in the environmental conditions *in vitro* and *in situ,* to which fibroblasts from MFH can contribute, similar to fibroblasts from the basal cell carcinoma [16].

Another example of an effect of the BFH-derived fibroblasts on the normal keratinocytes was provided by increased binding activity of the endogenous lectin galectin-1 to nuclei of keratinocytes under their influence. The expression of this lectin, a potent mediator of cell adhesion and tissue invasion as well as growth regulator by outside–inside signaling and intracellular target selection, e.g. to oncogenic H-ras [40–45], was also upregulated. To draw comparisons the stroma sections of basal cell carcinomas [16] and squamous cell carcinomas [46–48] as well as, interestingly, of psoriatic plaque [49] also have abundant presence of this lectin, and similar galectin-1-containing deposits are produced by stromal fibroblasts of basal cell carcinoma *in vitro* [16]. Thus, the production of this lectin and of sites with galectin reactivity are targets for factor(s) originating from stromal fibroblasts. This finding will aid the establishment of assays to define the nature of the effector molecules. When looking at the epidermal hyperplasia *in situ*, our results on the impact of BFH-derived fibroblasts in coculture, set in relation to results obtained in this system using basal cell carcinoma, appear to reflect the similar morphological status in the epidermis. Thus, the merit of the tested model appears to be underscored by the revealed similarities, warranting further work in this system.

Conflict of Interest Statement

All authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within that could inappropriately influence their work.

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