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Precision-cut slice cultures of tumors from MMTV-neu mice for the study of the ex vivo response to cytokines and cytotoxic drugs

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Abstract Ex vivo analysis of signaling pathways operating in tumor tissue is complicated by the three-dimensional structure, in particular by stroma–epithelial interactions. Studies performed with pure populations of tumor cells usually do not take into account this issue. One possibility to preserve the tissue architecture is the use of tumor slices. However, diffusion of oxygen and nutrients may become limiting factors, resulting in decreased cell viability and change of tissue morphology, especially after long-term incubation of slices. By using precision cut slices of defined thickness, we were able to establish culture conditions for tumor material obtained from MMTV-*neu* transgenic mice, which allow the study of the action of cytokines and cytotoxic drugs for up to 24 h. A slice thickness of 160 μm was found to be optimal for viability and handling of material. These slices were highly responsive to the action of the cytokine IFN-γ, as evident form the increase of pY701 STAT1, detected by both immunohistochemistry and western blotting, and by the increase of mRNA levels of the IFN-γ response genes *IRF-1*, *SOCS-1*, and *STAT1*, analyzed by reverse transcriptase–polymerase chain reaction. Furthermore, induction of apoptosis and increase of DNA damage could be monitored after treatment with IFN-γ or doxorubicin. The slices were also a convenient source for the establishment of explant cultures of tumor epithelial cells. It is concluded that cultivation of precision-cut tumor slices provides a convenient way for the ex vivo molecular analysis of MMTV-*neu* tumor tissue under conditions which closely simulate the situation in vivo and can provide an alternative to in vivo experiments.

Keywords MMTV-neu · Ex vivo drug testing · Stromal–epithelial interactions · Explant culture · Interferons

Introduction

Mice transgenic for the *erbB2*/*neu*/*HER2* oncogene serve as model systems for erbB2-induced breast cancer and have been extensively used to study mechanisms of tumor development, progression, as well as the response to tumor vaccination and cytotoxic drugs (Lollini et al. 2006; Ursini-Siegel et al. 2007). Experiments with these mice have been mostly performed in vivo but also with tumor cell lines derived from explant cultures of isolated tumors (Campbell et al. 2002). We were aiming to develop an ex vivo technique to study the response of the tumor to cytokines and cytotoxic drugs, which accounts for stroma–epithelial interactions and tissue architecture as important determinants for tumor development and progression (Shekhar et al. 2003; Tlsty and Coussens 2006; Kass et al. 2007).

In ex vivo experiments, two main strategies can be discerned to take this into account, namely, (a) reconstitution of the tissue organization using pure components of the tissue or (b) the cultivation of tissue pieces, also termed slice cultures (Kunz-Schughart 1999). Examples for reconstitutions are spheroid cultures formed by tumor epithelial cells and extracellular matrix components (Kunz-Schughart 1999) or the mammary adenocarcinoma model, which consists of a 1:1 mixture of tumor and stroma cells (Piechocki 2008). For preparation and cultivation of tissue pieces, various procedures have been developed, which vary in the size of the tissue pieces produced and the length

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of time the pieces are cultivated. One challenge encountered in experiments with tumor pieces or slices is the diffusion of nutrient and oxygen, which may become limiting in culture after withdrawal from blood circulation supporting the tissue in vivo. Only few studies using slice cultures have tried to monitor morphological and functional changes occurring in tissue slices after cutting and cultivation (Olinga et al. 1997; Toutain et al. 1998; Kern et al. 2006).

An important determinant for tissue viability is the thickness of the slices. This has been extensively studied for liver slices used in pharmaco-toxicological studies and has stimulated the development of instruments which allow the preparation of precision cut slices (Lerche-Langrand and Toutain 2000). In this report, we have used precision-cut slices of MMTV-*neu* tumors of various thickness and developed optimal experimental conditions for ex vivo studies, which preserve tissue integrity and cell viability. The optimized method was demonstrated to allow the study of the ex vivo action of IFN-γ and doxorubicin on tumor tissue using biochemical and histological methods.

Materials and Methods

Mice. Mice were bred and maintained in accordance with the Austrian animal welfare law and animal experiment act (BGBl. no. 501/1989 i.d.g.F). MMTV*neuN* transgenic mouse on FVB/N background (Guy et al. 1992) were purchased from Jackson Laboratory, Bar Harbor, Maine.

Mammary tumors. When the tumors grew to approximately 1-cm^3 size, mice were euthanatized by CO_2 inhalation, sacrificed by cervical dislocation, and tumors isolated by surgical excision. Collection of mammary tumors and transportation to tissue slicing facility was done after immersing tumors in ice-cold modified Kreb's–Hanseleit–HEPES buffer as described below.

Modified Krebs–Hanseleit buffer. Krebs–Hanseleit–HEPES buffer was prepared containing 120 mM NaCl, 4.8 mM KCl, 1.25 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 12.6 mM HEPES, pH 7.4. Buffer was further supplemented with 5 mM D-glucose and antibiotics (50 μg/ml gentamicin and 0.1% streptopenicillin), membrane-filtered (0.22-μm membrane; Steritop, Millipore, Marlborough, MA), and gassed with Carbogen (95% oxygen, 5% CO_2) for 30 min. This buffer was further referred to as modified Krebs–Hansleit buffer (MKHB). Mammary tumors were either fixed in 4% formalin or embedded in paraffin or snap-frozen and ground to powder in liquid nitrogen before further processing as described below.

Preparation of tumor slices. Whole mammary tumors were divided into approximately 0.5-cm^3 pieces with the sharp knife. Each tumor piece was embedded in the 4% SeaPlaque Agarose solution (FMC Corp., Rockland, ME) at 37°C using the tissue embedding unit (Alabama Research and Development Corporation, Munford, AL) in order to form agarose gel cylinders with a diameter of 16 mm. The cylinders were used to prepare precision-cut slices in a pre-cooled Krumdieck precision tissue slicer (model no. MD4000-01; Alabama R & D, Munford, AL) filled with oxygenated, ice-cold MKHB. This instrument has been designed to rapidly prepare aseptic, thin slices of live tissues for biochemical, pharmacological, toxicological, neurological, and other in vitro studies, an issue of critical importance also in the preparation of slices from tumors. Tumor slices were submersed in ice-cold MKHB until use. About 80 to 100 intact slices (160 μm) were obtained from a single 1-cm^3 tumor. The time required between excision of tumor and start of ex vivo incubation was 2 h or shorter.

Calibration of thickness of tumor slices. Krumdieck microtome settings for slice thickness were calibrated using a tissue micro-micromanipulator (Micromanipulator 5171, Eppendorf, Germany). For that purpose, tumor slices were spread on top of a glass slide and covered with a few drops of MKHB buffer. The micro-injector needle was directed to the upper surface of the tissue slice and then to the surface of the glass slide under visual guidance with a microscope and the number of 0.1-μm precision steps required by the micromanipulator to travel from the surface of the slice to the glass slide determined.

Ex vivo incubation of tumor slices. To avoid background problems due to the undefined levels of hormones, growth factors, and cytokines, serum-free medium was used for ex vivo experiments with tumor slices. Slices were loaded onto titanium grids in six-well plates (eight slices per well) containing 6 ml of Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 2.5% Albumax (GIBCO, Langley, OK), 50 μg/ml gentamicin, and 1% streptopenicillin. After treatment with or without IFN-γ (20 ng/ml; Peprotech. London, UK) or doxorubicin (1 μM; Sigma-Aldrich, St. Louis, MO), the slices were cultured in a rotating manner on an inclined plane in a humidified tissue culture incubator at 37°C. After the indicated incubation period, slices were either fixed in 4% buffered formalin (BF) or snap-frozen and ground to powder under liquid nitrogen for further histological or biochemical analysis.

Histological examination. Tumors and tumor slices were fixed in 4% BF and stored at 4°C for 24 h. After embedding in paraffin, 3-μm sections were made, deparaffinized, and

further processed for histochemical analysis as described below.

For tumor slice morphology, rehydrated sections were stained by hematoxylin and eosin (H&E) using standard procedures. The integrity of the tumor cells in the cultivated slices was monitored by comparison with control sections derived from fresh tumor material before slicing. The percent of damaged cells was calculated by counting the number of fragmented and/or shrunk nuclei over the total number of nuclei in three randomly selected fields for each tumor slice.

For immunohistochemistry, the antigens were retrieved in 10 mM citrate buffer, pH 6.0, at 98°C for 20 min. The slides were blocked in 5% horse serum for 1 h at room temperature. Primary antibodies and dilutions were as follows: pY STAT1, clone 58D61 (1:100, Cell Signaling Technology, Beverly, MA); cleaved caspase-3, clone 5A1 (1:400; Cell Signaling Technology); phospho-histone H2A.X (γH2A.X Ser139; clone 20E3; 1:100; Cell Signaling Technology). Immunoreactivity was detected by the Dako REAL EnVision Detection System (Dako, Glostrup, Denmark). Tissues were counterstained with hematoxylin. For visualization of apoptotic cells via the in situ terminal transferase-mediated dUTP nick end labeling method (TUNEL, Roche, Manheim, Germany), rehydrated slides of tumor sections were treated with 0.1% Triton X-100/ 0.1% sodium citrate buffer, dehydrated by graded series of alcohol and chloroform, and subjected to the TUNEL reaction in a humidified chamber for 1 h at 37°C using recombinant terminal transferase and fluorescein isothiocyanate-dUTP (Roche). The protocol provided by Roche was used. Counterstaining of nuclei was done with DAPI (Sigma-Aldrich).

Determination of nitrite concentration. After 48 h, nitrite was measured spectrophotometrically in the tumor slice culture medium by using Griess reagent as described (Schmidt et al. 1999).

RNA preparation and RT-PCR. Total RNA from tissue powder was isolated using the RNeasy kit (Qiagen, Hilden,

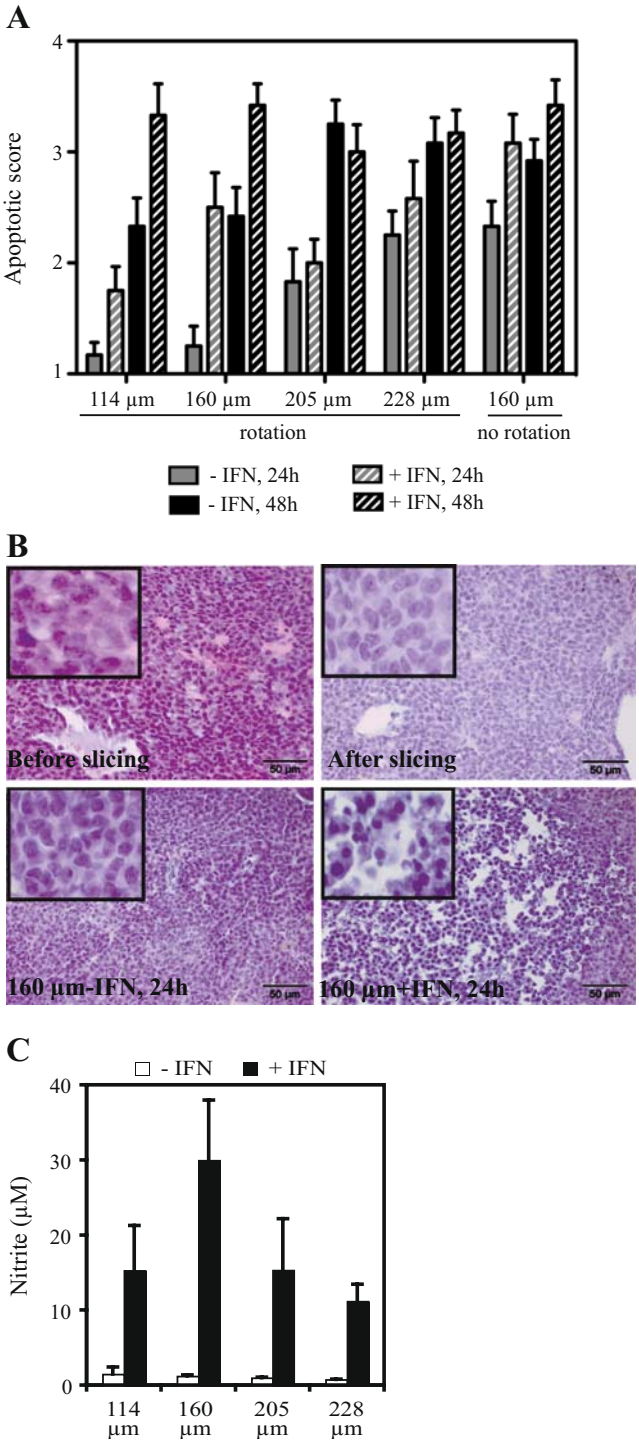

Figure 1. Morphological and functional changes of tumor slices after cultivation over a period of 48 h. Slices with the indicated thickness were cut and loaded onto titanium grids in six-well plates (eight slices per well) containing 6 ml of slice culture medium supplemented with or without IFN-γ (20 ng/ml). Slices were also cultivated in 24 well plates (one slice per well) without titanium grids containing 1 ml of slice culture medium supplemented with or without IFN-γ. Slices on titanium grids were cultivated in a rotating manner, whereas slices in 24-well plates were cultivated without agitation for 24 and 48 h. (*A*) Determination of apoptotic scores was performed on 3-µm sections of formalin-fixed paraffin-embedded slices stained with HE. A mean apoptotic score ranging from 1 to 4 was given to each section based on the percentage of cells with fragmented or shrunk nuclei. For each culture condition, four different slices were evaluated. The scores were defined as: less than 10% fragmented nuclei, score 1; 10% to 20 %, score 2; 20% to 50%, score 3; 50% and higher, score 4. (*B*) Representative pictures derived after H&E staining of the tumor slices cultivated with or without IFN-γ for 24 h are shown. (*C*) Nitrite concentration was measured in supernatants derived from each well after incubation of slices for 48 h with or without IFN-γ.

Germany), and RNA quality was evaluated by determination of ethidium bromide-stained 18S and 28S ribosomal RNA bands in an agarose gel. Reverse transcriptase–polymerase chain reaction (RT-PCR) was carried out with Superscript III reversely transcribed mRNA and the TaqMan protocol essential as described (Jurgeit et al. 2007). Amplification of the target genes was normalized to amplification levels of TATA box binding protein and to the levels of an appropriate control using the delta Ct method as described (Haffner et al. 2007). Sequences of primers and probes were used as following: mSTAT1; TCTTCCTGAACCCCCCGT (forward), GAAAACTGCC AACTCAACACCTC (reverse), CGCGTGGTGGTCCCA GCTCTC (probe); mIRF-1; GATGCCTGTCTGTTC CGGA (forward), TGGCTCTTTTTCTCCTGCTTTG (reverse), TGGGCCATTCACACAGGCCGA (probe); mSOCS1; TCCCTCTTAACCCGGTACTCC (forward), CTTAATGCTGCGGCACAGC (reverse), AGATCTGGAA GGGGAAGGAACTCAGGTAGTCA (probe).

Western blotting. Lysates from tissue powder were prepared at 4°C in co-immunoprecipitation lysis buffer (Haffner et al. 2008) by vigorously shaking for 60 min and centrifugation at 21,000×*g* for 20 min. Equal amounts of protein in the supernatants as determined with the Bradford method were boiled in sodium dodecyl sulfate (SDS) sample buffer and subjected to SDS polyacrylamide gel electrophoresis on 8% to 14% gels depending on molecular weight and the separated proteins transferred to Immobilion-FL membranes (Millipore). Membranes were incubated with primary antibodies overnight at 4°C and immunoreactive bands visualized using infrared dye conjugated antibodies and the Odyssey infrared imager (LICOR, Biosciences, Lincoln, NB). Quantification was performed using Odyssey application software version 2.1, provided by LICOR. Primary antibodies and dilutions were as follows: pY701-STAT1, no. 07-307 (1:1000, Upstate Biotechnology, Lake Placid, NY); STAT1, C-111 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA); cleaved

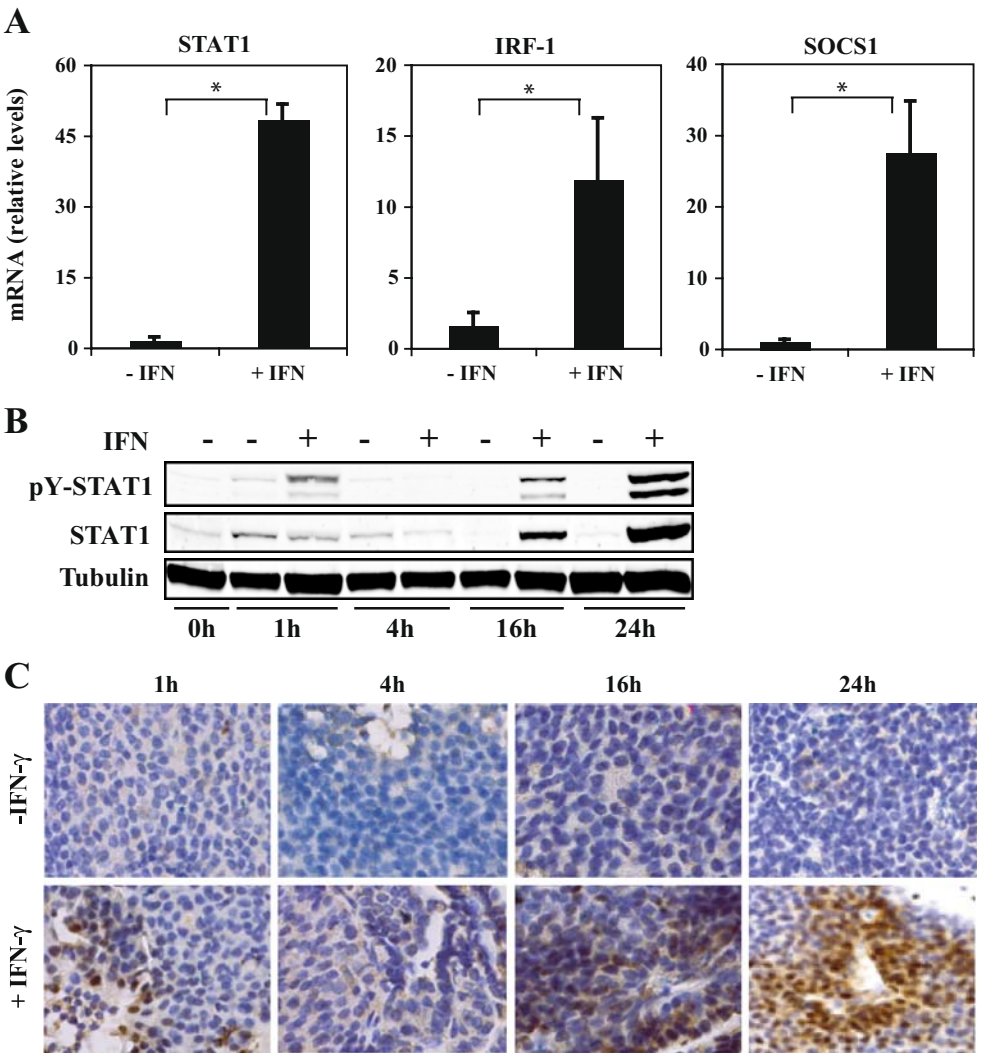

Figure 2. Monitoring of IFN-γ-dependent induction of gene expression in tumor slices. Slices were cultivated with 20 ng/ml IFN-γ for 24 h and further processed either by snap freezing and grinding to powder or by fixation in 4% formalin. RNA or whole cell lysates were prepared from tissue powder. (*A*) mRNA expression levels of IFN-γ responsive genes. Induction levels of SOCS1, IRF1, and STAT1 mRNA in response to IFN-γ were determined by RT-PCR (Taqman). *Error bars* represent mean±SEM of three independent experiments, $*p<0.05$. (*B*) Whole cell extracts from tissue powder of slices incubated with IFN-γ for 1, 4, 16, and 24 h were analyzed for STAT1 activation by immunoblotting with antibodies against tyrosine-phosphorylated (pY701) and total STAT1. (*C*) Three-micrometer sections from paraffin-embedded slices, obtained from the same experiment described in (*B*), were cut, deparaffinized, and immunostained using phosphorylated STAT1 (pY701). For (*B*) and (*C*), one representative experiment of three is shown.

caspase-3, clone 5A1 (1:1000; Cell Signaling Technology); phospho-histone H2A.X, clone 20E3 (1:1000; Cell Signaling Technology); α-tubulin, TAT1 (Woods et al. 1989; 1:2,000).

Explant cultures derived from MMTV-neu tumors. Explant cultures from MMTV-neu tumors were prepared according to Pei et al. (2004), with the modifications that precision-cut tumor slices obtained with the Krumdieck slicer were used. Slices were further cut into pieces with an area of approximately 2.5 mm^2. Thirty to 40 tumor pieces were placed on 10-cm tissue culture plates pre-coated with heat-inactivated fetal calf serum (FCS), covered with 2 ml of explant culture medium (DMEM/F12 supplemented with 0.5% heat-inactivated FCS, 200 mM L-glutamine, 5 μg/ml insulin, 10 ng/ml EGF, and 1 μg/ml hydrocortisone), and were kept at 37°C. Medium was changed every day. After 1 wk, outgrowth of cellular sheets with the morphology of epithelial cells was regularly observed by phase contrast light microscopy. A minority of tumor pieces exhibited outgrowth of fibroblasts. These pieces were immediately removed and contaminating fibroblasts in that area detached by trypsinization. After 2 to 3 wk, tumor pieces were removed and the outgrown tumor epithelial cells (TECs) collected by trypsinization. TECs were further cultivated in culture medium supplemented with 10% heat-inactivated FCS and used for up to five passages. TECs maintained the expression of cytokeratin K8 as epithelial marker and overexpressed neu at similar levels as found in the tumor (Fig. 5).

Results

Effect of slice thickness on tissue viability. Precision-cut tumor tissue slices were prepared using the Krumdieck tissue slicer. Tumors were all derived from animals with the same genetic background, namely, MMTV-*neuN* mice, strain N202, which develop mammary adenocarcinomas

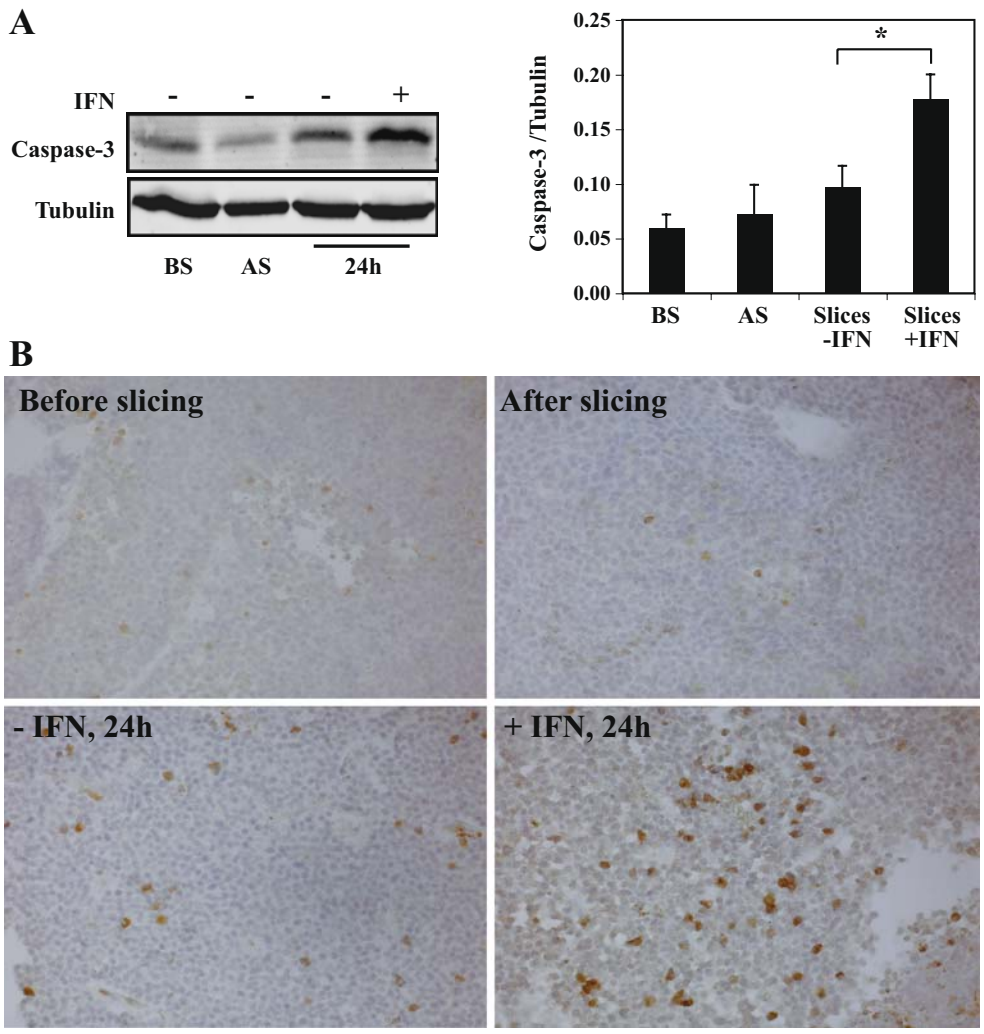

Figure 3. IFN-γ is inducing apoptosis in ex vivo cultivated tumor slices. Results obtained by Western blotting and immunohistochemistry are shown for tumor tissue derived before slicing (*BS*), directly after slicing (*AS*), or for slices incubated with or without 20 ng/ml IFN-γ for 24h. (*A*) Determination of caspase-3 cleavage product (17 kDa) in slices by Western blot. In the *left panel*, a representative Western blot is shown. The *right panel* gives the quantification of four independent experiments (*$p<0.05$; *bars* represent mean±SEM). (*B*) Immunohistochemical staining of slices fixed in 4% formalin using an antibody against active caspase-3.

with a mean latency of 205 d (Guy et al. 1992). We used slices with a thickness ranging from 114 to 228 μm in our study and incubated them for up to 48 h at 37°C in culture medium. Slices smaller than 100 μm were very difficult to handle and had a strong tendency to disintegrate during incubation. Slices with thickness of 114 μm were possible to handle, but still were very fragile. The effect of slice thickness, incubation conditions, and incubation time on tissue viability was tested by determining the percentage of tumor cells with damaged nuclei in H&E-stained sections. These data were used to calculate a relative apoptotic score (Fig. 1*A*). Before slicing and directly after slicing, less than 1% of cells exhibited damaged nuclei (see Fig. 1*B*, upper two panels for representative micrographs). After incubation for 24 h under rotating conditions to facilitate diffusion of nutrients and oxygen, slices with a thickness of 114 and

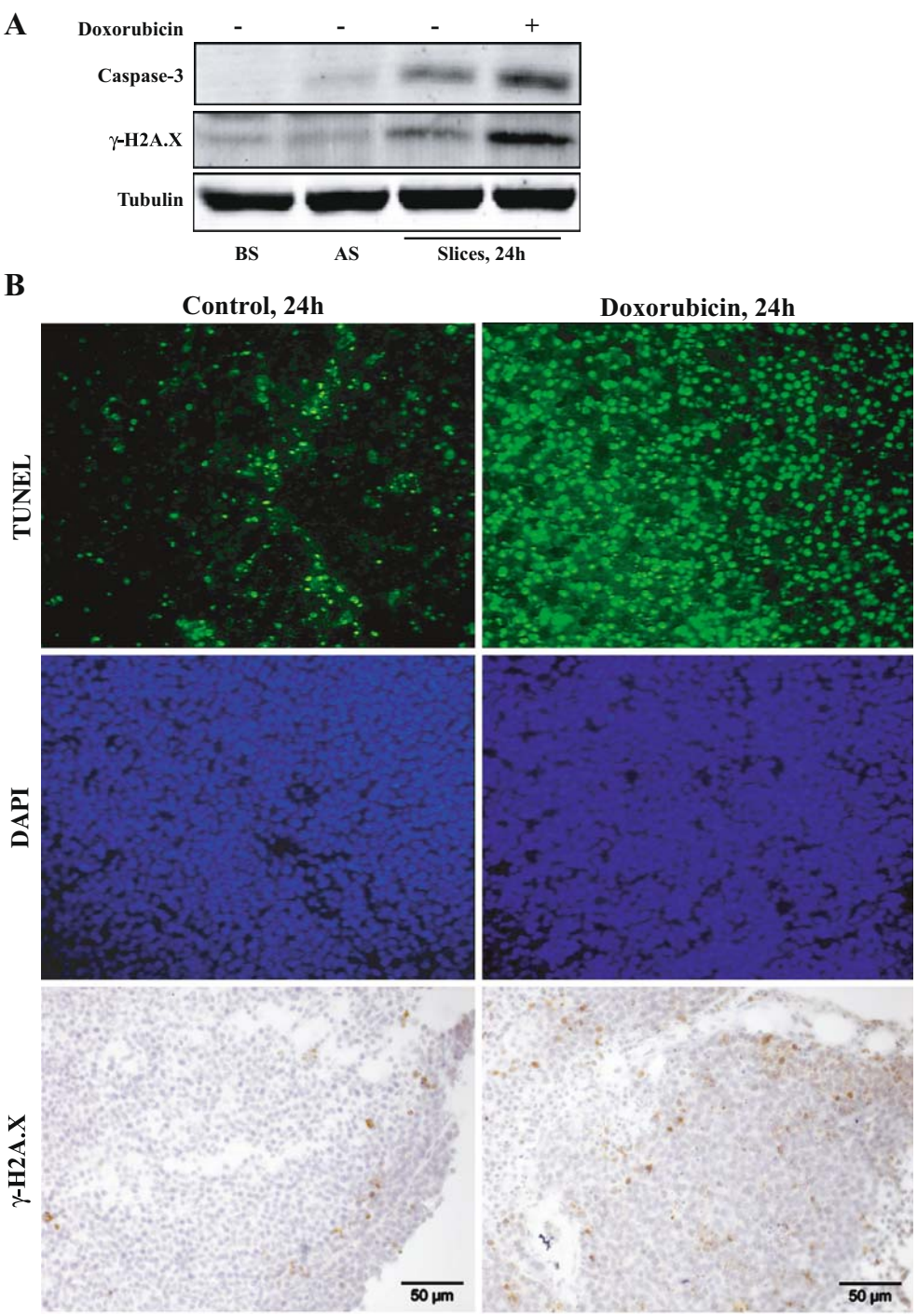

Figure 4. Doxorubicin-induced cell death in slice cultures. Slices were treated ±1 μM doxorubicin. After 24 h, slices were either snap-frozen or ground to powder or fixed in 4% formalin. (*A*) Tumor cell death was monitored by detection of cleaved product of caspase-3 (17 kDa) and the phospho-form of γ-H2A.X using Western blot. Doxorubicin significantly increased the abundance of caspase-3 cleavage product and γ-H2A.X in slice cultures ($p<0.05$). (*B*) DNA damage produced by doxorubicin was further evaluated in formalin-fixed slices by staining of TUNEL-positive nuclei (*green*, *upper row*) with the matching DAPI staining (*blue*, *middle row*) or by monitoring of γ-H2A.X phosphorylation (*lower row*).

160 μm exhibited the lowest frequency of damaged nuclei, whereas in 205- and 228-μm-thick slices, the percentage was higher and significantly different as compared to 114-μm slices ($p<0.05$). Inclusion of IFN-γ in the incubation medium significantly increased the apoptotic score for the 114- and 160-μm slices only ($p<0.05$, compare also lower panels of Fig. 1*B* for representative results obtained with 160-μm slices). Incubation for 48 h resulted in high apoptotic scores for all four thicknesses (Fig. 1*A*). When slices were incubated without rotation, high apoptotic scores were already observed for 160-μm slices even after 24-h incubation in culture medium without IFN-γ (Fig. 1*A*, last four bars). We conclude that for the different conditions tested, incubation of 160-μm slices for up to 24 h on rotating grids is best suited for the experiments, since slices of this thickness are easy to handle, show low apoptotic score when incubated in culture medium alone, and maintain the capacity to respond to IFN-γ treatment. These slices also exhibited the highest capacity to produce nitric oxide in response to IFN-γ as judged by the increase of nitrite concentration in the culture medium (Fig. 1*C*). Therefore, further experiments were all performed under these conditions.

Tumor slices allow the monitoring of IFN-γ effects on STAT1 signaling and induction of apoptosis. We further tested the suitability of our slice culture system to monitor effects of IFN-γ with biochemical and immunohistochemical techniques. As shown in Fig. 2*A*, analysis of RNA derived from IFN-γ-treated slices revealed a strong increase of mRNA specific for STAT1, which is upregulated after STAT1 activation (Levy and Darnell 2002), and the STAT1 target genes *IRF-1* and *SOCS1*. Figure 2*B*, *C* demonstrates that already after 1 h of stimulation, activation of STAT1 was detectable by Western blotting and by immunohistochemistry using an antibody specific for STAT1 pY701. Furthermore, activation of caspase-3 was detectable with an antibody specific for the 17-kDa cleavage product, both in Western blot assays (Fig. 3*A*) and by immunohistochemistry (Fig. 3*B*).

Tumor slice cultures can be used to study the action of cytotoxic drugs. Doxorubicin is a cytotoxic drug that produces cell death by inducing DNA strand breaks and is commonly used to treat breast cancer (Kurz et al. 2004). We evaluated its effect on tumor cell death and in exerting genotoxic stress. A significant activation of caspase-3 and increased phosphorylation of histone γ-H2A.X as a marker for DNA damage was observed in Western blot experiments after incubation of slices with 1 μM doxorubicin (Fig. 4*A*, $p<0.05$). Increase of apoptosis and DNA damage was also observable by TUNEL reaction ($p<0.01$) and histone γ-H2A.X immunohistochemistry (Fig. 4*B*).

Precision-cut tumor slices facilitate the establishment of explant cultures. When freshly cut slices were seeded on FCS-coated culture tissues as described in "Materials and Methods," outgrowth of TECs was detectable by phase contrast light microscopy after 1 wk (Fig. 5*A*). As shown in Fig. 5*B*, these TECs retained the epithelial marker protein cytokeratin 8 and the expression and activation of the tumor promoting protein erbB2, as judged by comparison of the abundance of Y877 phosphorylated erbB2 and total erbB2 in the initial tumor and in TECs.

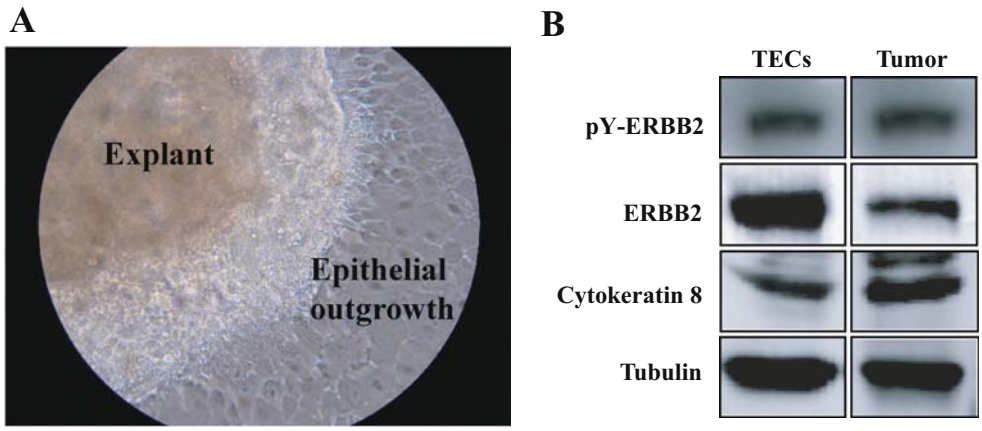

Figure 5. Precision-cut MMTV-neu tumor slices can be efficiently used to prepare explant cultures of tumor epithelial cells (*TECs*). (*A*) Typical epithelial outgrowth from explanted slices as observed 1 wk after cultivation. (*B*) Subcultures of TECs retain the expression status of the original tumor from which they were derived for phospho-erbB2 (pY 877), erbB2, and cytokeratin-K8 proteins. After trypsinization of primary explants cultures, TECs were further sub-cultured for up to five passages and expression and phosphorylation of proteins in the original tumor and the explants compared by Western blot.

Discussion

In this report, we describe precision-cut slice cultures as a novel procedure to perform ex vivo experiments on tumors of MMTV-*neu* transgenic mice, which preserves the three-dimensional structure of the tumor and provides an interesting alternative to in vivo experiments. Thickness of slices was found to be a critical issue. Optimal thickness is determined by a compromise between handling of tissue, which is better with thicker slices and short distances for diffusion of nutrients and oxygen, which requires thin slices. In the case of mammary adenocarcinomas from MMTV-*neu* mice, a slice thickness of 160 μm was best suited to allow both good handling and diffusion distances short enough to ensure tissue viability in long-term cultures. Similar as observed with liver slices (Toutain et al. 1998), rotating cultures were superior to multi-well plate cultures and better prevented tissue damage. In liver slices, this was attributed to a better oxygen supply of the tissue in rotating cultures (Toutain et al. 1998). Optimal thickness appears to be dependent on the tissue investigated (Parrish et al. 1995), indicating the requirement for optimization for each different type of tissue.

Cultures of tissue slices from MMTV-*neu* mammary tumors exhibited increased cell death and frequency of apoptotic cells when incubated longer than 24 h. This appears to be different when compared to slice cultures of prostate tissue, which can be maintained over several days in culture (Parrish et al. 2002; Blauer et al. 2008) and human breast-cancer-derived slices, which were possible to use up to 4 d for drug testing (van der Kuip et al. 2006). Cervix (Kendrick et al. 2008) and ovary (Kendrick et al. 2007) carcinoma tissue slices were reported to tolerate incubation times of 48 h without strong effects on tissue viability. In the case of non-small cell lung cancer, an increase of the frequency of dead cells was already observed after 16 h (Lang et al. 2007).

Slice cultures can also be infected with viruses. They have been used to study therapeutic efficacy and toxicity of conditionally replicative adenovirus (Kirby et al. 2004) and might be also useful for virus-mediated gene transfer. The problem faced in such experiments is the limited capacity of viruses to penetrate into tissues, and usually, only the surface layers of the tissue are reached by the virus (Kolodkin-Gal et al. 2008). Thus, the preparation of ultra-thin slices appears to be mandatory for this type of experiments to ensure a high yield of infected cells in the slices. With 114-μm-thin slices of MMTV-*neu* tumors, which is the lower thickness limit compatible with handling, infection yields of up to 30% of the tissue could be obtained (our unpublished results).

As shown in Fig. 5, precision-cut slice cultures also represent a convenient source for the establishment of explant cultures of tumor epithelial cells, since the ultra-thin slices have high surface area allowing efficient outgrowth of tumor epithelial cells at the rate which is superior to the one observed with tumor pieces obtained with conventional cutting procedures.

Reproducibility represents an important issue for ex vivo studies performed with tissue sections obtained from an individual animal or patient. One reason for that are differences in the genetic background between individuals. In animal experiments with mice, the problem can be minimized by the use of inbred strains of genetically modified mice. For this reason, we only used MMTV*neuN* transgenic mice on FVB/N background in our study. This allowed the repetition of ex vivo experiments with tumor slices using material derived from mice with identical genetic background.

In conclusion, precision-cut slice cultures prepared from tumors of transgenic mice represent a powerful tool to study the role of three-dimensional structure and stroma–epithelial interactions in tumor biology and the response of a particular tumor type to environmental cues, drugs, and cytokines under well-defined and reproducible conditions. It thereby well complements other techniques based on the use of cell lines for the reconstitution of tissue organization.

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