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A case report on malignant phyllodes tumour with bone metastasis demonstrating DNA damage response and organoid guided sensitivity to doxorubicin

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Case Report

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Abstract

Malignant phyllodes tumours are rare breast neoplasms with limited systemic treatment options, particularly in the metastatic setting. In this case report, malignant phyllodes tumour was diagnosed in a 61-year-old female who subsequently developed a metastasis to the femur two years after initially presenting with isolated primary breast disease. The patient presented with an isolated osseous lesion and underwent an open biopsy and frozen section of the femur via a lateral approach. Secondary bone malignancy consistent with previous breast pathology was confirmed on frozen section. Subsequently, meticulous intralesional curettage was performed, followed by intramedullary femoral nailing and cementation of the surrounding bone defect. A sample of the resected tumour was cultured ex vivo as patient-derived explants (PDEs) and as 3-dimensional patient-derived organoids (PDOs). PDOs showed selective response to treatment with doxorubicin, compared to paclitaxel or eribulin. Although the patient was not eligible for chemotherapy, such PDO analysis could ultimately be helpful as a precision medicine approach to identify treatment options for patients. We detected extensive γH2AX staining, as an indicator of DNA damage, consistent with the sensitivity seen to DNA damaging agents such as doxorubicin. Our data demonstrate the potential utility of ex vivo analysis in a personalised approach to treatment, particularly in the PDOs, which showed a better viability profile compared to PDEs.

Introduction

There is an increasing need for application of precision medicine to treat diseases due to varied lifestyle, diet, climatic conditions, host microbiota, immune make up and genetic susceptibility among individuals (1). In precision oncology, organoids are generated from tissues harvested from individual patient tumours and are commonly known as patient-derived organoids (PDOs). Patient-derived explants (PDEs) are architecturally intact ex vivo models of surgically removed tumours that are tested for drug efficacy soon after their removal, without the dissociation of the tumour. Both PDOs and PDEs have the potential to recapitulate the patient response to specific agents (2–5).

Phyllodes tumours are rare fibroepithelial tumours that account for less than 0.5% of all breast neoplasms. Phyllodes tumour is most commonly diagnosed in women aged 40 to 50 years old (6) and rarely occurs in men (7). WHO recognizes 3 types of phyllodes tumours: benign, borderline and malignant. Of the three recognised subtypes, malignant phyllodes tumours account for only 10–15%, highlighting their relative rarity and clinical significance (8). Histologically, malignant phyllodes tumours are distinguished by their degree of marked stromal atypia, marked stromal cellularity, stromal overgrowth, high mitotic count, infiltrative tumour border and malignant heterologous elements. They manifest a potential for local recurrence (up to 30%) and more rarely, distant metastatic spread (1.7% – 16%), with features associated with distant metastatic spread including tumour size greater than 7 cm, marked stromal overgrowth, marked cellularity, infiltrative borders and necrosis (9).

The mainstay of management for malignant phyllodes is surgical excision with at least a 1cm margin. There is a role for radiotherapy in local disease control but without evidence for survival benefit.

Chemotherapy has been used for recurrent and metastatic disease, but phyllodes tumours do not usually respond well to common therapies (8). The estimated 5-year survival rate for malignant phyllodes tumours ranges between 54% – 82%. We present here a malignant phyllodes case study to exemplify a functional precision medicine approach that could potentially inform treatment selection.

Case Presentation

A 61-year-old female smoker with no other known significant medical history presented with a large (560 g), well-encapsulated right breast mass involving approximately 60% of breast volume. Wide local excision and sentinel node biopsy identified a 110 mm malignant phyllodes tumour with necrosis, stromal hypercellularity, stromal overgrowth, infiltrative borders, moderate cytologic atypia and frequent mitoses; margins were positive, but nodes were negative. Staging CT showed no metastases, and although mastectomy was recommended, the patient initially declined. Margins were re-excised and cleared, but the tumour recurred within two months as an 8 × 10 cm mass beneath the nipple–areolar complex, requiring mastectomy four months after initial surgery. The mastectomy specimen showed a 75 mm recurrent malignant phyllodes, histologically similar to the first tumour. Immunohistochemistry (IHC) revealed positivity for DOG1, CD10, SMA, bcl2, vimentin and patchy TLE1, and negativity for p63, GATA3, CD117, ER, SATB2, CK8/18, desmin. Due to delayed wound healing, adjuvant radiotherapy was not commenced, and chemotherapy was not recommended.

Eighteen months later, she sustained a pathological fracture of the left femur. Initial frozen section was nondiagnostic and temporary fixation was performed. After formal histology confirmed metastatic malignant phyllodes, she underwent intralesional curettage, retrograde intramedullary nail fixation and cementation (Fig. 1A). She consented to inclusion in the CPAC ex vivo culture study (HREC/2020/QRBW/61294; QUT2000000363).

Post-operative radiotherapy was delivered once wound healing permitted. The metastatic lesion was a high-grade malignant spindle-cell neoplasm with abundant apoptosis infiltrating bone trabeculae, morphologically matching the breast tumour (Fig. 1B&C). Both breast and bone lesions shared identical immune profiles (positive DOG1, CD10, SMA, bcl2, vimentin, p53; negative MUC4, GATA3, GCDFP15, ER, PR, CD34, EMA, AE1/AE3, STAT6, CD117, S100, MyoD), with focal CK8/18, p63, desmin staining only in the bone metastasis.

Five months later, FDG-PET identified a pathological fracture of the left humeral head, FDG-avid lesions in the right ilium and right femoral neck, and three subcutaneous nodules in the left thigh.

She underwent right hip joint replacement and further radiotherapy to multiple sites. She died ~ 1 year after surgery for her initial osseous metastasis. A summary of the clinical course and key interventions is provided in Table 1.

Table 1
Chronological timeline of clinical presentation, disease progression, and therapeutic interventions in the patient

Timepoint	Clinical highlight(s)
Initial presentation	Large right breast mass (~ 60% breast volume); biopsy confirmed malignant phyllodes tumour
Initial surgery	Wide local excision and sentinel lymph node biopsy; positive margins
2 weeks post-surgery	Margin re-excision; margins cleared
2 months post-initial surgery	Local recurrence (8 × 10 cm mass)
4 months post-initial surgery	Right mastectomy performed
~ 18 months post-mastectomy	Pathological fracture of left femur; diagnosis of bone metastasis
Subsequent femur surgery	Curettage, intramedullary nailing, and cementation
Post-operative period	Radiotherapy to femur following wound healing
5 months later	FDG-PET showed further metastases (humerus, ilium, femoral neck, subcutaneous nodules)
Subsequent treatment	Hip replacement and radiotherapy to multiple metastatic sites
Final outcome	Patient admitted to palliative care and died ~ 1 year after initial bone metastasis

Pathological Findings

A sample of the metastatic tissue (925 mg) was processed for histology, fresh-frozen storage, PDOs, and patient-derived explants (PDEs). PDEs (1–2 mm^3 slices) were cultured on gelatin sponges (Spongostan™) pre-soaked with organoid medium (10) in 24-well plates at 37°C, 5% CO_2, 21% O_2, with media changed every 5 days.

PDE viability was assessed by MTS assay at days 0, 2, 7, 12. Absorbance at 495 nm was quantified after incubation in phenol-red-free RPMI1640 (Corning Inc.) plus MTS reagent (Promega Corp.). Tissues were fixed at day 12, processed to FFPE, and analysed with H&E, Ki-67, and cleaved caspase-3 (CC3) staining.

Initial PDEs showed high proliferative activity (Ki-67) and morphology consistent with parent tumour tissue, followed by a progressive decline in metabolic activity and loss of structural integrity by day 12, concordant with reduced Ki-67 and increased CC3 staining (Fig. 2).

Tissue obtained from the bone metastasis resection was dissociated into single cells as previously described (11) and cultured in the breast PDO media described by Sachs et al (12) with 10% Matrigel

(Corning Inc.) on ultra-low adhesion (ULA) 24-well plates (Costar®). Once formed, PDOs were seeded into 96-well ULA plates (Costar®) and treated with eribulin (0.1 μM; Eisai Inc.), paclitaxel (0.5 μM; Selleck Chemicals) and doxorubicin (0.1 and 10 μM; Selleck Chemicals). Following 72 hours of treatment, the PDOs were imaged using phase-contrast microscopy (Fig. 3A–E). Subsequently, they were encapsulated in 1% agarose and processed into formalin-fixed paraffin-embedded (FFPE) specimens for histological analyses, including H&E staining. Of the chemotherapeutic drugs tested, only doxorubicin resulted in PDOs exhibiting pronounced disaggregation (Fig. 3D&E). Loss of organoid structure and morphology is indicative of induced cell death.

We next evaluated both the source tissue, as well as PDOs and PDEs derived from the clinical specimen, to confirm phyllodes phenotype. PDOs were resuspended and encapsulated in 1% agarose, sectioned and stained for tumour marker expression. Immunohistochemistry for DOG1 (13) and CD10 was performed on FFPE clinical tumour specimen, PDEs and PDOs. As shown in Fig. 4, immunohistochemistry demonstrated consistent expression of DOG1 and CD10 across all sample types.

Having confirmed DOG1 expression is maintained in PDO cultures, we next evaluated the tumour tissue for potential mechanisms contributing to the observed PDO doxorubicin sensitivity. As genetic insult is a primary doxorubicin mechanism of action, we examined expression of the DNA damage marker γH2AX in the phyllodes source tumour. As shown in Fig. 5, immunohistochemistry analysis identified extensive γH2AX staining in the phyllodes tumour, suggesting elevated genome instability in the tumour. Given the detectable measure of DNA damage, these data provide a possible mechanism for sensitivity to DNA damaging agents such as doxorubicin and point to the precision medicine potential for PDOs.

Discussion

Precision medicine has become a cornerstone of cancer therapeutics. Personalised treatment aims to deliver more efficient and effective treatments for cancer patients and reduced tissue morbidity by informing the delivery of the right treatment to each individual patient at the right time. To facilitate establishing a precise treatment regimen, it is important to test the likely effectiveness of the treatment without harming the patient. This challenge could be overcome by testing various drugs by generating PDEs or PDOs with donor tissues.

Here we demonstrate the utility of a precision medicine approach for a malignant phyllodes tumour case, which is an uncommon fibroepithelial lesion of the breast with varied potential for malignant transformation (14). For this case, tumour recurrence occurred within 2 months of wide local excision, with the presence of metastases ultimately confirmed after bone fracture ~ 18 months later. Though complete mastectomy was performed in the third surgery, an early removal of the breast may have prevented the subsequent distant metastasis in the bone. Even so, this is an unusual manifestation for phyllodes tumour, and a classic example that not all cancers, though clinically alike, behave in a similar fashion in all patients.

In the case of malignant phyllodes tumour, while early aggressive surgery is key, effective systemic therapies are lacking. Chemotherapy remains the final line of treatment for stage IV disease, and precision medicine approaches hold promise for identifying suitable therapeutic options and informing treatment selection. To evaluate our precision medicine platform, we treated PDOs with several common chemotherapeutics. Our findings demonstrated an improved response to doxorubicin compared with the anti-microtubule drugs paclitaxel and eribulin. Doxorubicin exerts its antineoplastic effects predominantly through induction of DNA damage via DNA intercalation, which serves to halt genome replication and topoisomerase enzyme inhibition (15). While recent multi-omic analyses demonstrate genomic measures of instability in malignant phyllodes tumours (5, 16, 17), to the best of our knowledge, we are the first to demonstrate elevated measures of functional DNA damage via γH2AX staining in a malignant phyllodes tumour. In keeping with γH2AX as a functional marker of response for DNA damaging chemotherapeutics (18, 19), our findings point to a molecular understanding for why the PDOs, at least for this specific malignant phyllodes, were responsive to doxorubicin compared with agents with anti-mitotic mechanisms of action.

Our PDO results align closely with findings from Chu et al. (20), who similarly demonstrated that malignant phyllodes PDOs retain the phenotype of their source tumours and show strong sensitivity to doxorubicin but limited response to paclitaxel. While their cases showed additional CD34 and c-Kit staining, our metastatic and primary tumours shared a consistent profile (DOG1, CD10, SMA, Bcl2, vimentin, p53) with focal CK8/18, p63 and desmin only in the bone lesion. Overall, our immunohistochemical and drug-response patterns parallel those reported by Chu et al., further supporting the relevance of PDOs in modelling therapeutic sensitivity in malignant phyllodes tumours.

Unfortunately, the patient succumbed to the disease due to distant metastasis and systemic complications. Therefore, the PDO results inferred in the lab could not be translated to the clinic for this patient. However, our results provide corroborative evidence to demonstrate that PDOs from malignant phyllodes tumours could be helpful in identifying the ideal chemotherapeutic agent to treat the tumour as well as prevent its progression, and Chu et al had the same conclusion (20). In a precision medicine context, these two studies demonstrate the applicability of PDOs within the management of malignant phyllodes tumours, which might help guide clinical decision making to identify responsive systemic therapies for improved patient health outcomes.

In conclusion, we demonstrate the potential utility of PDOs to provide an indication of chemosensitivity, and of DNA-damage assessment by γH2AX staining as an indicator of sensitivity to DNA-damaging therapies.

Abbreviations

CC3
Cleaved caspase 3
FFPE

Formalin-fixed paraffin-embedded
H&E
Hematoxylin and eosin
IHC
Immunohistochemistry
PDE
Patient-derived explant
PDO
Patient-derived organoid
ULA
Ultra-low adhesion

Declarations

Ethics approval and consent to participate

This study was approved by the Queensland University of Technology Human Research Ethics Committee (HREC approval number: HREC/2020/QRBW/61294; QUT2000000363) and conducted in accordance with the Declaration of Helsinki. Patient-derived tissues used for organoid and explant modelling were obtained following appropriate ethical approval and informed consent.

Consent for publication

Written informed consent was obtained from the patient for publication of this report and any accompanying images or clinical data.

Competing interests

The authors declare no competing interests.

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Author Contribution

S.G.S.: Conceptualization of manuscript, writing – original draft and revision. T.v.W.: Specimen collection, writing – review and editing. P.L.J.: Investigation. A.T.: Investigation. S.N.: Investigation. A.A.:

Investigation. L.P.: Investigation, visualization. K.O.: Writing – review and editing. D.L.: Specimen collection, clinical resources, writing – review and editing. K.S.T.: Specimen collection, clinical resources, writing – review and editing. V.A.: Specimen collection, clinical resources, writing – review and editing. I.B.: Specimen collection, clinical resources, writing – review and editing. E.D.W.: Writing – original draft, formal analysis, visualization, writing – review and editing. M.N.A.: Formal analysis, data curation, visualization. P.D.R.: Conceptualization, supervision, resources. E.W.T.: Funding acquisition, conceptualization, supervision, investigation, formal analysis, data curation, visualization, writing – review and editing. All authors have read and approved the final version of the manuscript.

Data Availability

All data generated or analysed during this study are included in this published article. Additional information is available from the corresponding author on reasonable request.

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Figures

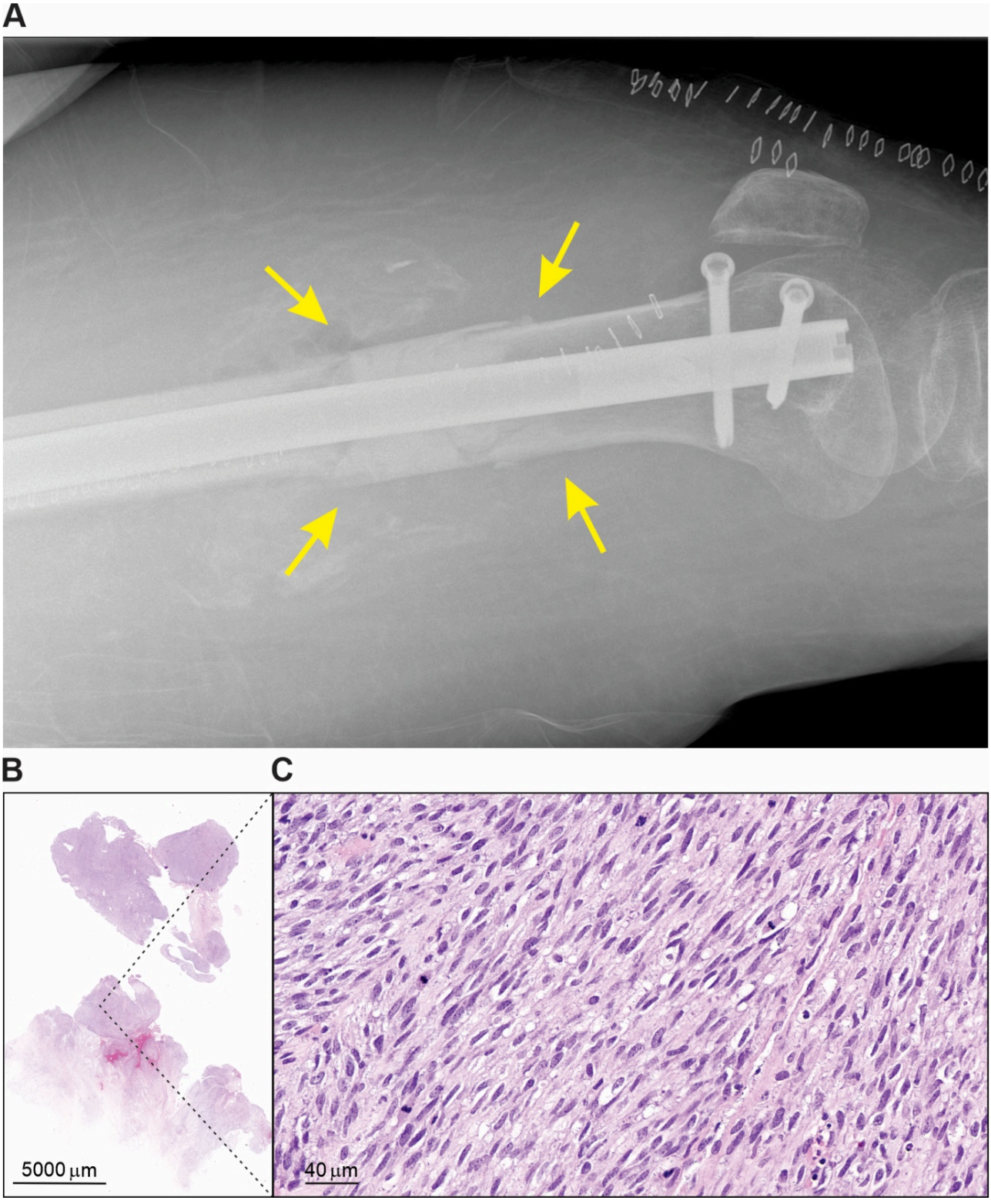


Figure 1

Radiograph showing the fractured site in the left femur (yellow arrows) and placement of intramedullary nails and cementation for rehabilitation (A). Hematoxylin and eosin (H&E) staining of original bone metastasis tissue (B, C). Scale bars are included within the images for each section shown.

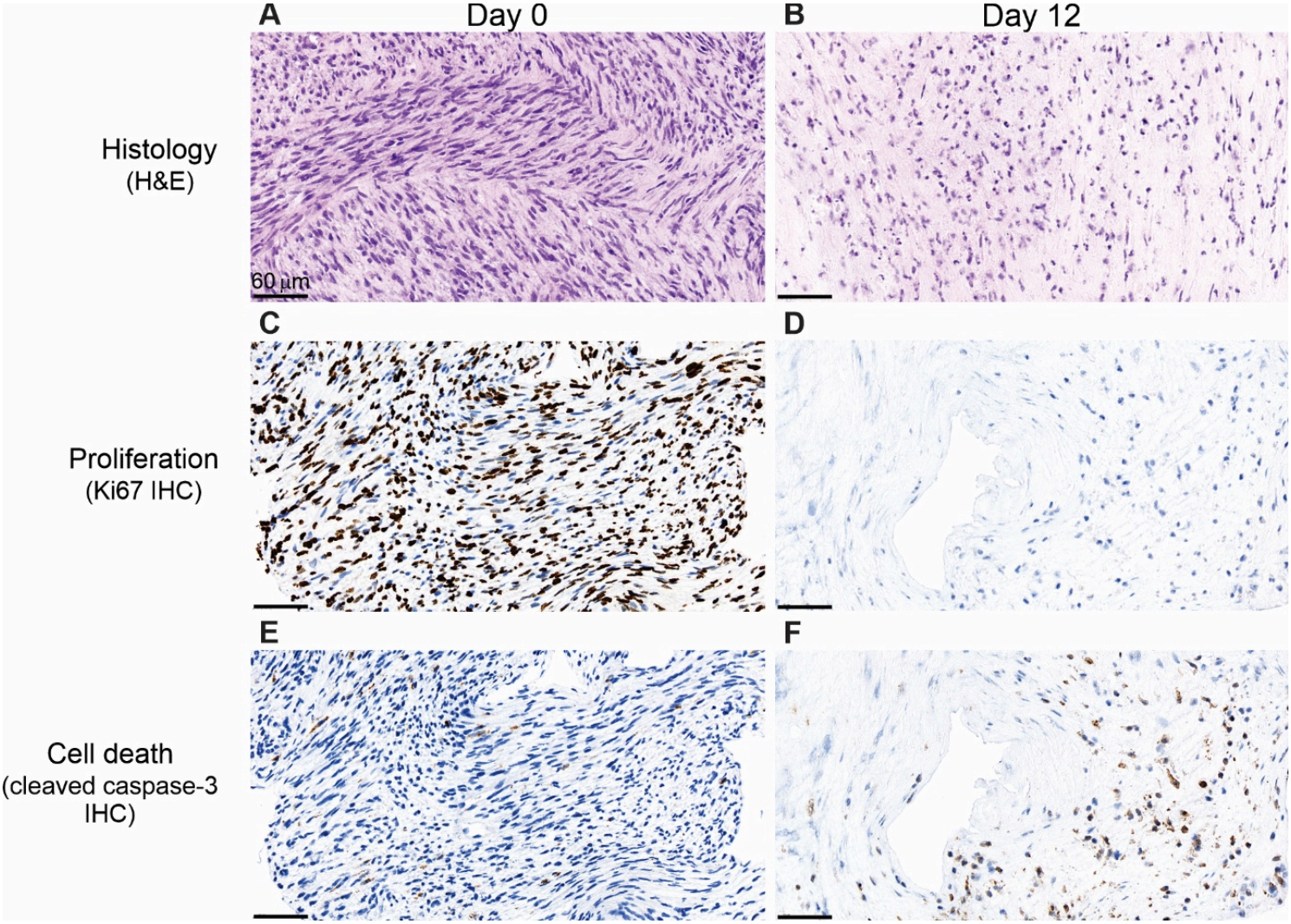


Figure 2

PDE tissues from metastatic tumour showing decreased viability at the end of the culture period (on Day 12) compared to Day 0 via H&E staining (A, B). IHC staining of Day 0 and Day 12 PDEs for proliferation marker Ki67 (C, D) and apoptosis marker CC3 (E, F). Brown nuclei on IHC sections represent positively stained cells. Scale bar shown in (A) applies to all images.

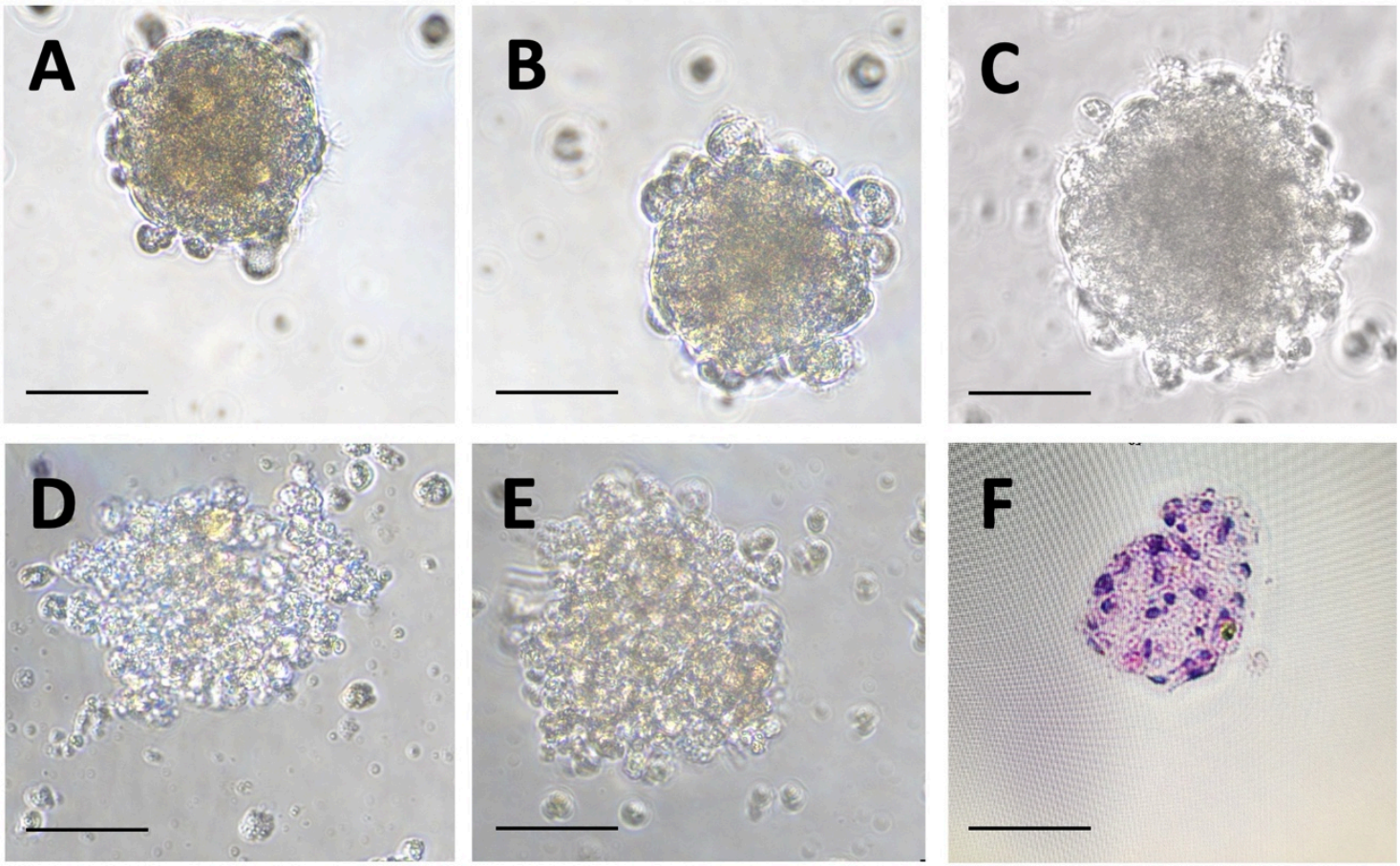


Figure 3

Morphological (A-E) and histological (F) analyses of PDOs treated for 72 hours with (A) vehicle control (dimethyl sulfoxide; DMSO), (B) paclitaxel (0.5 μM), (C) eribulin (0.1 μM), (D) doxorubicin (0.1 μM) and (E) doxorubicin (10 μM). (F) Haematoxylin and eosin-stained section of agar embedded control organoid. Scale bars - 50 μm.

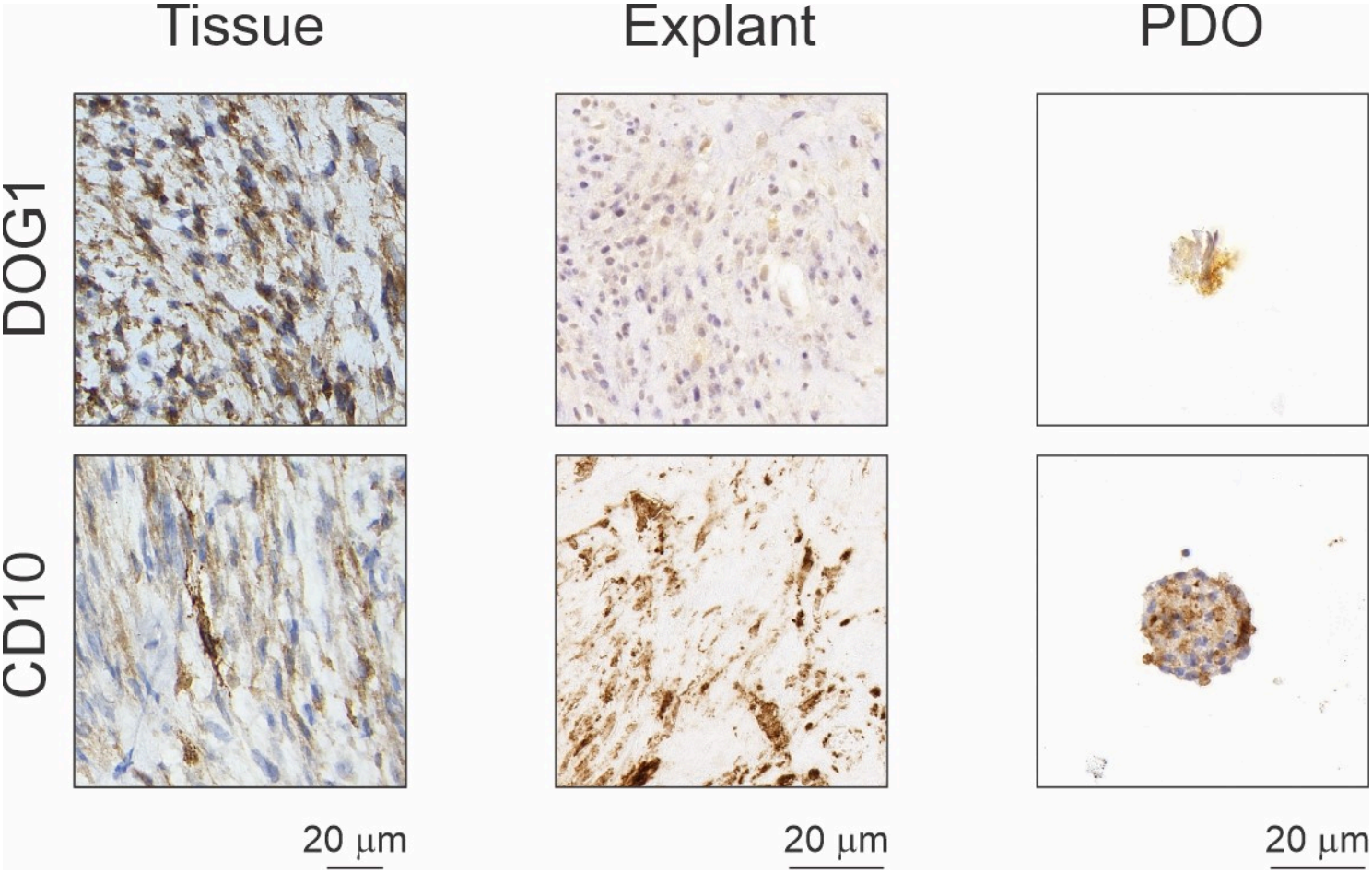


Figure 4

Representative immunohistochemistry images demonstrating DOG1 and CD10 positivity in phyllodes tumour tissues (left panel), explant cultures (centre panel), and PDOs (right panel). Scale bars noted below images.

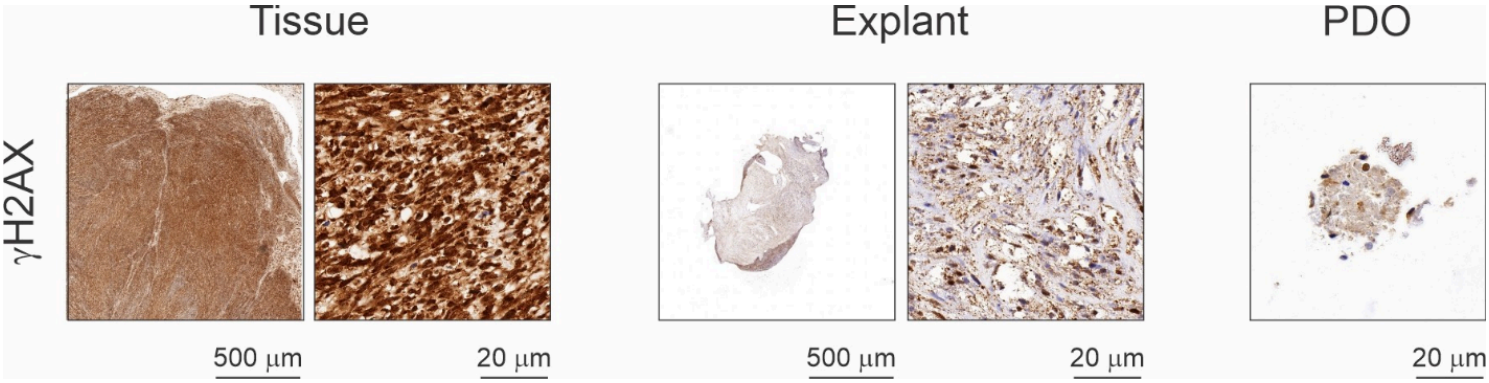


Figure 5

γH2AX immunohistochemistry showing strong signal in phyllodes tumour tissue (left panel), explant culture (centre panel), and PDO (right panel). Scale bars noted below images.

Supplementary Files

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