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Establishment and characterization of a novel cell line derived from human thymoma AB tumor

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Thymomas are low-grade epithelial tumors of the anterior mediastinum. The complexity of the disease and the lack of *in vitro* and *in vivo* models hamper the development of better therapeutics. In this study, we report a novel cell line, designated as IU-TAB-1, which was established from a patient with stage II thymoma (World Health Organization-type AB). The IU-TAB-1 cell line was established *in vitro* and characterized using histological and immunohistochemical staining, fluorescence-activated cell sorting, cytogenetic analyses and functional assays including *in vitro* and a NOD/SCID xenograft model. A whole-genome gene expression analysis (Illumina) was performed on the IU-TAB-1 cell line and 34 thymomas to determine the clinical relevance of the cell line. The IU-TAB-1 cell line was positive for epithelial markers (pan-cytokeratin and EpCAM/CD326) including thymic epithelial (TE) surface markers (such as CD29, CD9, CD54/ICAM-1, CD58 and CD24) and p63, and negative for B- and T-cell lineage markers. Gene expression profiling demonstrated overlapping and distinct genes between IU-TAB-1 and primary thymomas including the primary tumor (from which the cell line was derived). IU-TAB-1 cells are tumorigenic when implanted in immunodeficient mice with tumors reaching a volume of 1000 mm^3 at around 130 days. The established cell line represents a biologically relevant new tool to investigate the molecular pathology of thymic malignancies and to evaluate the efficacy of novel therapeutics both *in vitro* and *in vivo*.
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Thymomas are rare epithelial tumors of the thymus gland with diverse pathological findings and clinical outcomes. The classification of these tumors is controversial and the commonly used World Health Organization (WHO) unifying schema categorizes thymic tumors into thymomas (subtypes A, AB and B1–B3) and thymic carcinomas (formerly grouped as type C).[1,2] The current therapeutic management is almost entirely based on tumor stage.[3] Although complete resection and chemotherapy are effective therapies for early disease, metastases remain a challenge.[4–6] Tumors of all histological types, albeit with different frequencies, can spread locally within the pleura and pericardial cavity, and give rise to local or distant metastases.[5,6] The treatment strategies include the combination of surgery, radiation and chemotherapy based on the extent of the disease and the status of tumor resectability.[3,4] Surgery is the main choice of treatment for localized thymic tumors, as complete resection is the most significant prognostic factor. In patients with advanced thymic malignancies, cisplatin and carboplatin-based combination chemotherapy has become standard.[7,8] Though remissions may be of significant duration, chemotherapy alone is not felt curative with relapses in unresectable patients being almost universal. Patients with locally advanced and metastatic thymoma have a reduced disease-free survival. Second cancers and associated immunological disorders also contribute to decreased overall survival of these patients.[9]

Given the rarity of the thymic epithelial (TE) neoplasms, new treatments are based on the studies extrapolated from other epithelial tumors rather than biology-driven therapeutic strategies. No mechanistic studies exist to predict the unique predilection for pleural disease recurrence and

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metastasis, or the development of second malignancies. Recent comprehensive genomic analysis has shown molecular distinctions between different histological types of thymic tumors.[10] Although targeted therapies directed against epidermal growth factor receptor (EGFR) and KIT are available,[11,12] but these, unlike in lung cancer, do not appear to be efficacious (PJL, unpublished data). Preclinical evaluation of efficacy of novel therapies is limited due to the lack of representative cellular and animal models. Among the four cell lines reported to date, three were isolated from thymic carcinoma patients,[13–15] while one cell line was established from type B1 thymoma.[14] To the best of our knowledge, this is the first human spindle cell thymoma cell line derived from a patient with type 'AB' thymoma. We further explored its clinical relevance in comparison with the primary thymic tumors, and our studies support its usefulness as a preclinical tool in TE malignancies.

MATERIALS AND METHODS

Brief Clinical Summary of the Patient

A 53-year-old male with 35 pack-year history of smoking presented with an upper respiratory infection. Chest radiograph revealed a 6.5-cm anterior mediastinal mass. He did not have any history of radiation exposure or symptoms of paraneoplastic syndromes, including myasthenia gravis. At operation, the tumor was found to be adherent to the lung and pericardium hence an *en bloc* thymectomy with partial pericardectomy and wedge resection of the right upper lobe was performed. Pathology revealed a thymoma of WHO AB type with microscopically invasion of adjacent adipose tissue. The tumor, in all the 13 sections examined, was composed of spindle cells admixed with variable number of lymphocytes (B-like areas). A separate type B-cell component was not identified. All resection margins, peritumoral lymph nodes and the adherent lung were negative for tumor (stage II thymoma (T2N0M0)). Adjuvant radiotherapy was considered but not given and the patient was disease-free at the time of last follow-up. As the patient did not have signs or symptoms of immune-mediated illness or recurrent infections a detailed immunological evaluation was not performed.

Establishment of the Cell Line

An IRB-approved written informed consent was obtained from the patient to conduct research with his resected tumor. A piece of sterile tumor sample from this patient was used to establish the cell line. The tumor was cut into small pieces in a sterile 100-mm tissue plastic dish. Tissues were cultured in RPMI-1640 medium (Mediatec, Manassas, VA, USA) supplemented with 5% FBS, 1% penicillin and streptomycin in a 5% CO_2 atmosphere at 37 °C. The medium was changed every 4 days. To remove fibroblasts, we applied a number of methods including keeping the serum concentration at 5% FBS, selective trypsinization and adhesion techniques as described by Langdon.[16] After 2 weeks, cells were transferred into 75-cm^2 flasks. The flasks were regularly assessed for epithelial cells outgrowth. The pure population of epithelial cells was designated as IU-TAB-1.

Immunohistochemistry of the Primary Tumor and the Established Thymoma Cell Line, IU-TAB-1

Cells were examined by light microscope using phase contrast and methylene blue staining according to the manufacturer's instructions (Fisher Scientific, Pittsburgh, PA, USA). Cytological preparations and cell blocks were made for the cells and a comparative analysis was performed with the primary tumor using hematoxylin and eosin (H&E) morphology and a number of immunohistochemical stains (CD1a, CD20, CD99, TdT, p63, EGFR and c-KIT). The antibodies were purchased from Dako (Carpinteria, CA, USA) and immunohistochemistry was performed according to the manufacturer's instructions.

Immunophenotype by Flow Cytometric Analysis

Expression of surface markers was assessed using fluorochrome-conjugated-specific antibodies followed by flow cytometry analysis. The following antibodies were used: FITC-conjugated CD3, CD8, CD9, CD10, CD13, CD15, CD16, CD18, CD19, CD21, CD34, CD40L/CD154, CD45, CD49b, CD49d, CD49e, CD54/ICAM-1, CD90 and CD100; PE-conjugated CD4, CD6, CD24, CD29, CD38, CD40, CD49a, CD49c, CD49f, CD50, CD51/CD61 ($\alpha v\beta 3$) CD58, CD62L, CD95, CD106, CD117, CD133, CD134, CD138, CD140a/PDGFRα, CD140b/PDGFRβ, CD142, CD146, Ep-CAM/CD326, NG2, EGFR and FLT3. Antibodies were obtained from BD Biosciences (San Jose, CA, USA), BD/PharMingen (San Diego, CA, USA) or Beckman Coulter (Miami, FL, USA). Cells were labeled for 30 min at 4 °C; irrelevant isotype-matched antibodies were used as negative controls. Samples were acquired in a BD FACSCalibur EPC flow cytometer, and at least 10 000-gated events were acquired for each sample.

Chromosome Analyses

G-banded karyotyping was performed on IU-TAB-1 cell line (passage 10). Dividing cells were prepared for conventional cytogenetic analysis following standard protocols. Cells were dropped onto cold, wet glass slides and then aged at 90 °C for 60 and 20 min at 65 °C followed by GTG banding. In total, 20 dividing cells were analyzed. Karyograms were prepared with an imaging system (Genetix, Santa Clara, CA, USA).

Microarray Analysis

RNA isolation and whole-genome—cDNA-mediated annealing, selection, extension and ligation (WGDASL; Illumina, San Diego, CA, USA) of IU-TAB-1 (quadruplets for the cell line) and 34 thymomas were performed according the manufacturer's instructions. The probe set used was the Illumina Human Ref-8BeadChip. This BeadChip features up to date content covering $>24\,000$ annotated genes derived

Table 1 Chromosome aberrations detected in IU-TAB-1 cell line in comparison with other histological types

Genetic aberrations	Histological types reported	IU-TAB-1
1q gain	B2–B3 and thymic carcinoma;[10,23,24] A, AB[21,22]	Not identified
1q–1q21 and 1p	AB and all types[10,21,22]	Present
2	AB and all types[22,23]	Present
3	AB and B2–B3 and thymic carcinomas[10,21–23,24]	Present
4p gain	B3 and thymic carcinoma[10]	Not identified
4	AB and all types[22,23]	Present
5	AB and all types[21–23]	Present
5q21–22	B2 and invasive B3[21,24]	Not identified
6p21.3	All types[21–24]	Not identified
r(6)	AB[33]	Not identified
6q23–q25	AB and all types[10,21,23]	Present
7p15	B2, B3, thymic carcinoma and some AB thymomas[21]	Present
8p11.21	B2, B3, thymic carcinoma[21]	Not identified
8p21	AB and B types[21,23]	Present
8q11	B2 and B3	Not identified
9	A, AB, B1[22,23]	Not identified
9p loss	A, thymic carcinoma[22]	Not identified
10p	AB[21]	Present
11q21–23	AB[21]	Present
12p	B2, B3[22]	Not identified
13q	AB and all types[10,21–23]	Present
13q14.3	B2, B3, thymic carcinoma and some AB thymomas[21]	Not identified
16p gain	A, B2[22]	Not identified
16q	AB and all types[21,22,24]	Present
16q22.1	B2, invasive B3, thymic carcinoma[21,24]	Not identified
(16;12)(q11;p11.2)	AB[34]	Not identified
17p gain	A, AB[22]	Not identified
17p13 loss	Invasive B3 and thymic carcinoma[10,21,22,24]	Not identified
17q gain	B3 and thymic carcinoma and some AB[10,22,24]	Not identified
17q	AB[21,22]	Present
18q gain	B3 and thymic carcinoma[10,24]	Not identified
18q	AB and B types[22,23]	Present
20	A, B2, B3[22]	Present
22	A[22]	Present

from RefSeq (build 36.2, release 22). The clinical details of the patients were described in Table 1.[17] The tumors were categorized into three groups: group I ($n=9$)-type AB; group II ($n=19$)-types B1–2; and group III ($n=6$)-type B3. Briefly, data on 34 patients from Illumina Human WGDASL arrays, with each array containing 18 401 probes were analyzed. Genes that had a poor signal quality across a maximal number of arrays were filtered out. As a result, 8260 genes were found to have signals significantly above background. The samples were run in three batches and batch effect was removed statistically using Partek Genomics suite's batch effect removal tool. The data were quantile normalized and $\log_2$ transformed before statistical analysis. After quantile normalization, unsupervised hierarchical clustering was performed. In addition, comparisons were made between cell line, original tumor sample and other primary tumors for TE cell surface antigens,[18] stromal gene signatures[19] and immune-system-related genes.[20]

In vitro Growth Properties of IU-TAB-1 Cell Line

Cell growth was assessed both by trypan-blue dye (Sigma, St Louis, MO, USA) exclusion and bromodeoxyuridine (BrdU) cell proliferation method (Roche Diagnostics, Indianapolis, IN, USA). IU-TAB-1 cells were plated as 0.5×10^6 cells in a 60- mm plate (trypan blue) or 8000 cells/well in a 96-well plate, and allowed to attach overnight. Cell viability (trypan blue dye exclusion) and cell proliferation (BrdU cell colorimetric ELISA kit) were measured at 24, 48 and 72 or 96 h according to the manufacturer's instructions.

Matrigel Morphogenesis Assay

Growth factor-reduced Matrigel (BD Biosciences) was plated in 48-well plates and incubated at 37 °C for at least 1 h to allow for its gelation. IU-TAB-1cells (3×10^4) were seeded on the Matrigel, and incubated at 37 °C overnight (o/n). Cultures were observed at 6, 12, 24 and 48 h, and photographed using a Nikon D40X camera.

Thymoma Xenograft Model

Five- to six-week-old NOD/SCID mice (obtained from the *In Vivo* Therapeutics Core at Indiana University Melvin and Bren Simon Cancer Center) were acclimatized for 6 days. IU-TAB-1 cells (10×10^6 cells/mouse; six mice per group) mixed with Matrigel were injected subcutaneously into their flanks and allowed to set. Cells were also injected into flanks without Matrigel. Tumors were measured weekly using caliper for external measurements. Tumor volume was calculated as $L \times W^2/2$, where L is length and W is width. All animal experiments were done under a protocol approved by the Indiana University Institutional Animal Care and Use Committee.

RESULTS

IU-TAB-1 Cell Line is TE in Origin

The primary tumor showed a pattern of type AB histomorphologically composed of spindle cells admixed with variable number of lymphocytes (B-like areas; Figure 1a). To confirm the epithelial nature of the IU-TAB-1 cells, we performed

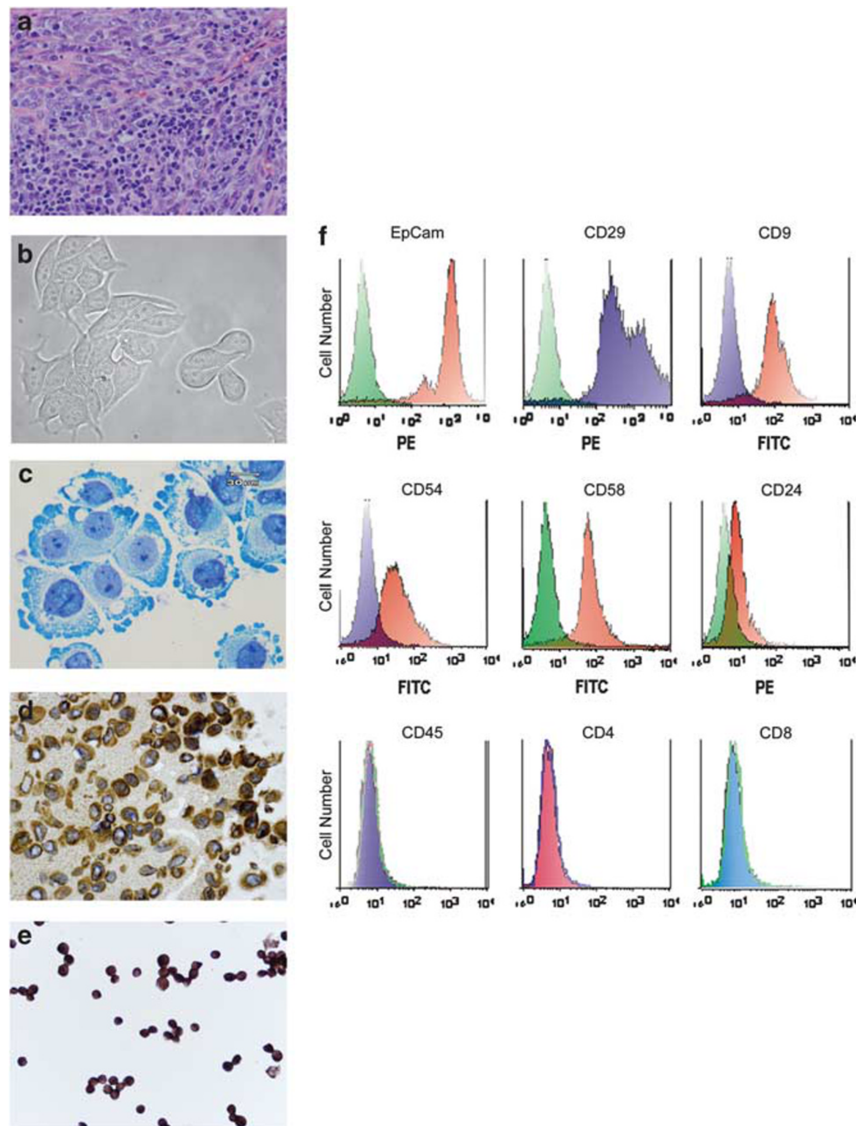


Figure 1 Morphology and origin of the IU-TAB-1 cell line are shown in (**a**) representative hematoxylin and eosin staining of the primary thymic tumor from which IU-TAB-1 cells were derived. (**b**) High-power magnification (× 40) of the cultured cell line under microscope. (**c**) Methylene blue staining of the cultured cell line under microscope. (**d**) Immunohistochemical staining with positivity for pan-cytokeratin, (high-power magnification × 40). (**e**) Immunohistochemical staining with nuclear positivity for p63 (high-power magnification × 40). (**f**) Flow cytometry analysis for surface expression of EpCAM, CD29, CD9, CD54, CD58, CD24, CD45, CD4 and CD8.

morphological assessment, immunohistochemical staining and fluorescence-activated cell sorting (FACS) analysis. IU-TAB-1 cells grew as adherent monolayer with a cobblestone morphology characteristic of epithelial cells (Figure 1b). Methylene blue staining further verified the high nucleic acid content of the cell line with prominent nucleoli (Figure 1c). The cells were positive for pan-cytokeratin (Figure 1d), p63 (Figure 1e), and EpCAM/CD326 (Figure 1f) and negative for the lymphoid and other leukocyte markers, including CD45, and T-cell markers including CD4 and CD8 (Figure 1f). Importantly, the cells expressed CD29, CD9, CD58 and CD24 that have been shown to be common between TE cells and epidermal keratinocytes and CD54/ICAM-1 is positive only in TE cells. The rest of the markers listed in Materials and Methods were negative. These results confirm that IU-TAB-1 cell line is TE in origin. The cells, as well as the original tumor, were also negative for TdT, CD99, CD1a, c-KIT and EGFR. The tumor, but not the cell line, was focally positive for CD20 (data not shown).

Most of the Chromosome Aberrations Listed in Type AB Thymomas are Present in IU-TAB-1 Cell Line

We next assessed whether IU-TAB-1 cell line contains some of the chromosome aberrations reported earlier. Table 1 summarizes the genetic aberrations present in IU-TAB-1 in comparison with other type AB and histological types

reported in the literature. Among them, aberrations in 6q23q25, 7p15, 8p, 13q and 16q were common in all thymoma types including type AB.[21–24] Other aberrations listed in type AB thymomas include 2, 3, 4, 5 and 18, which were present in IU-TAB-1 cell line. On the other hand, IU-TAB-1 cell line did not have the aberrations of 5q21-22(APC), 13q14.3 (RB) and 17p13.1 (p53) that were specific to a subset of more invasive type B3 thymomas.[21,24,25] IU-TAB-1 exhibits some other aberrations reported in AB cases such as1q, 10p, 11q21–q23, 13q, 16q and 17q. These results support the relevance of IU-TAB-1 cell line as a useful preclinical model.

Relevance of IU-TAB-1 Cell Line in Comparison with the Primary Thymic Tumors

To investigate the relevance of IU-TAB-1 cell line to the primary thymomas, a whole-genome gene expression analysis was performed on the IU-TAB-1 cell line, and compared with 34 frozen thymomas categorized into three groups as described in Materials and Methods. Unsupervised hierarchial clustering revealed two main branches and seven subgroups (Supplementary Figure S1). All the IU-TAB-1 replicates clustered together. Of the nine AB tumors, six of them were in the same branch including the IU-TAB-1 patient. The remaining three AB samples were clustered in the second branch group.

To further assess the similarities and dissimilarities between IU-TAB-1 cell line and its primary tumor of origin or other thymomas, we aimed for specific gene sets that may distinguish TE tumor cell line from the complex tumor microenvironment of the primary tumors (Figures 2a–d). Of the genes related to the cell surface antigens in resting and interferon-γ-treated human TE cells identified by Patel *et al*,[18] 21 surface antigens were differentially regulated in the different groups (Figure 2a). More specifically, CD9, CD24 and CD58 were strongly upregulated in IU-TAB-1 cell line, confirming its TE nature.

To further exclude stromal origin of IU-TAB-1 cells, we compared the cell line and tumors based on their stromal content. We compared our gene expression profiles with two distinct stromal signatures reported in Webster *et al.*[19] These signatures consist of a core set of fibroblast genes (a core set of 66 desmoid-type fibromatosis (DTF)-associated genes; DTF fibroblast signature correlated with lower tumor grade) and of a macrophage response core gene set (a core set of 112 genes—CSF1signature associated with higher tumor grade). Importantly, there was a marked downregulation of these stromal genes from both signatures in IU-TAB-1 cells when compared with the primary tumors. This further confirms the epithelial phenotype of the cell line (Figures 2b and c). As expected, differential regulation of these genes were observed in primary tumors. Interestingly, most of the group II tumors (B1–B2) clustered in the branch with high expression of these macrophage response genes, whereas group I representing AB tumors were in the low-expression branch.

To analyze the expression levels of the immune-system-related genes in IU-TAB-1 and primary tumor groups, we used the list of 199 Affymetrix ProbeSets categorized into 7 metagene clusters by Rody *et al.*[20] The immune-system-related genes were mostly downregulated in IU-TAB-1 cell line, while they were differentially regulated between different groups of primary tumors (Figure 2d). Interestingly, the tumor origin of IU-TAB-1 was among the samples with very low expression of immune-system-related genes. Most of the immune-response-related genes were upregulated in group II representing B1–B2 thymomas. Taken together, these results suggest that IU-TAB-1 cell line is representative of TE cells and do not express stromal- and immune-related genes.

The Functional Growth Properties of IU-TAB-1 Cell Line *In Vitro* and *In Vivo*

To determine the *in vitro* growth kinetics and doubling time of IU-TAB-1 cell line, we used both trypan-blue dye exclusion method and BrdU cell proliferation assay (Figures 3a and b). The doubling time was calculated as 48 h (Figure 3a). The morphogenic properties of IU-TAB-1 cells were evaluated by analyzing these features in Matrigel cultures. After incubation for 12 h, active cell locomotion was observed in Matrigel, with the formation of luminal-like clusters, and with some expansion of the clusters over time (Figure 3c). These clusters resembled the structures formed in Matrigel by luminal MCF-7 or ZR75-1 breast cancer cells, and were distinct for the cellular networks formed by more invasive mesenchymal-like breast cancer cells (as MDA-MB-231 cell line).

To assess whether IU-TAB-1 cells were capable of forming tumors in immunodeficient NOD/SCID mice, cells were implanted subcutaneously admixed with Matrigel or alone. As shown in Figure 3d, IU-TAB-1 cells are tumorigenic, forming ectopic tumors that reached a volume of 1000 mm^3 at around 130 days when in Matrigel-plugs, and around 148 days when implanted alone. Importantly, the kinetics of tumor development is consistent with the indolent nature of AB thymomas. The mice were killed and paraffin-embedded sections were prepared to stain with H&E and pan-cytokeratin as an epithelial cell marker. The malignant epithelial nature of xenograft IU-TAB-1 tumor was confirmed histologically (H&E; Figure 3e) and immunohistochemically (pan-cytokeratin and p63) (Figures 3f and g). This demonstrates that IU-TAB-1 line contains cells with tumor-initiating properties.

DISCUSSION

Preclinical models have been important tools to understand the molecular pathology of human cancers and to develop better therapeutics. Various human cancer cell lines isolated from patient tumors have provided extensive information for the cellular and molecular mechanisms of cancer biology. For example, the development of multiple breast cancer cell lines and xenograft models contributed to our understanding of the molecular targets in breast cancer tumorigenesis and

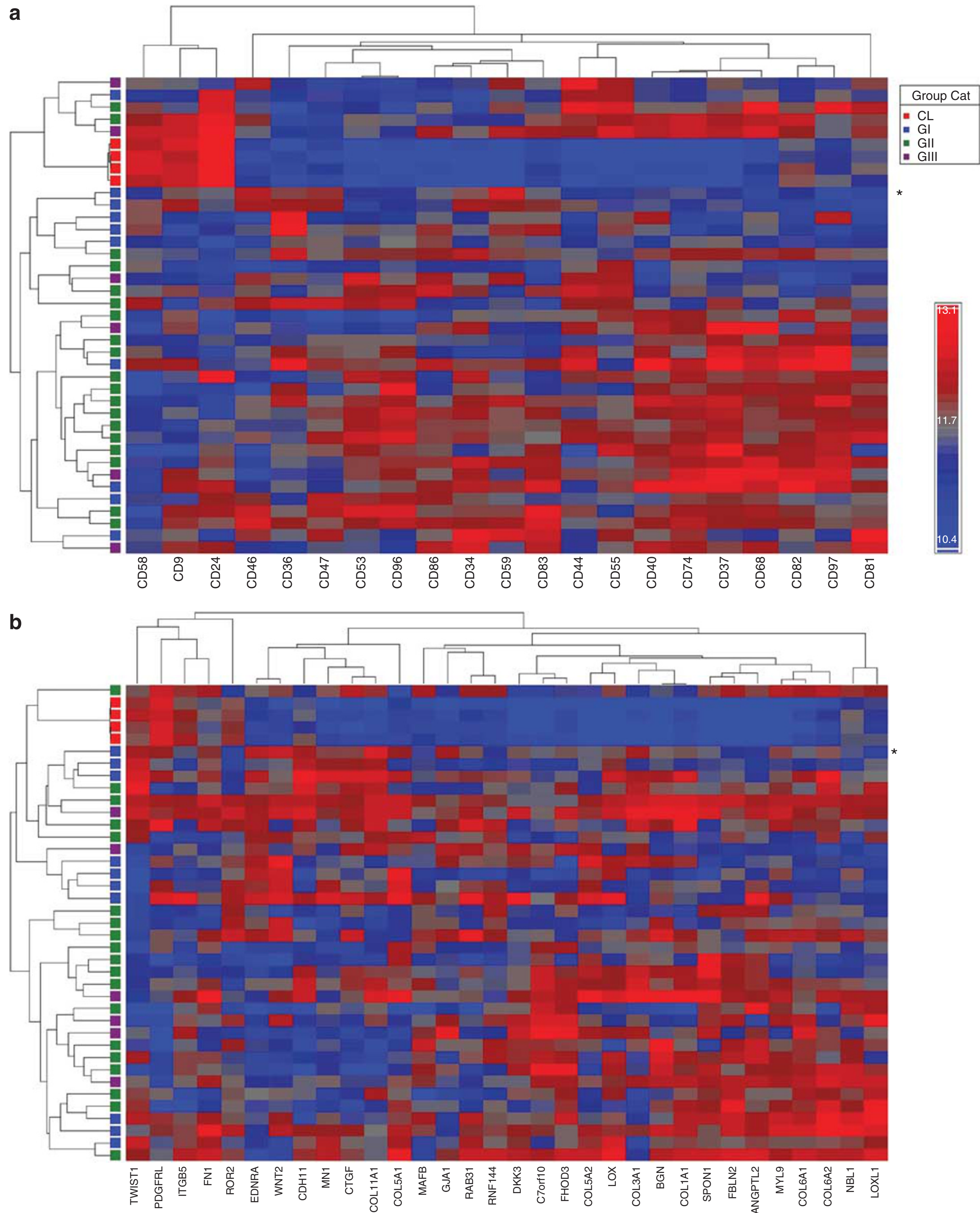
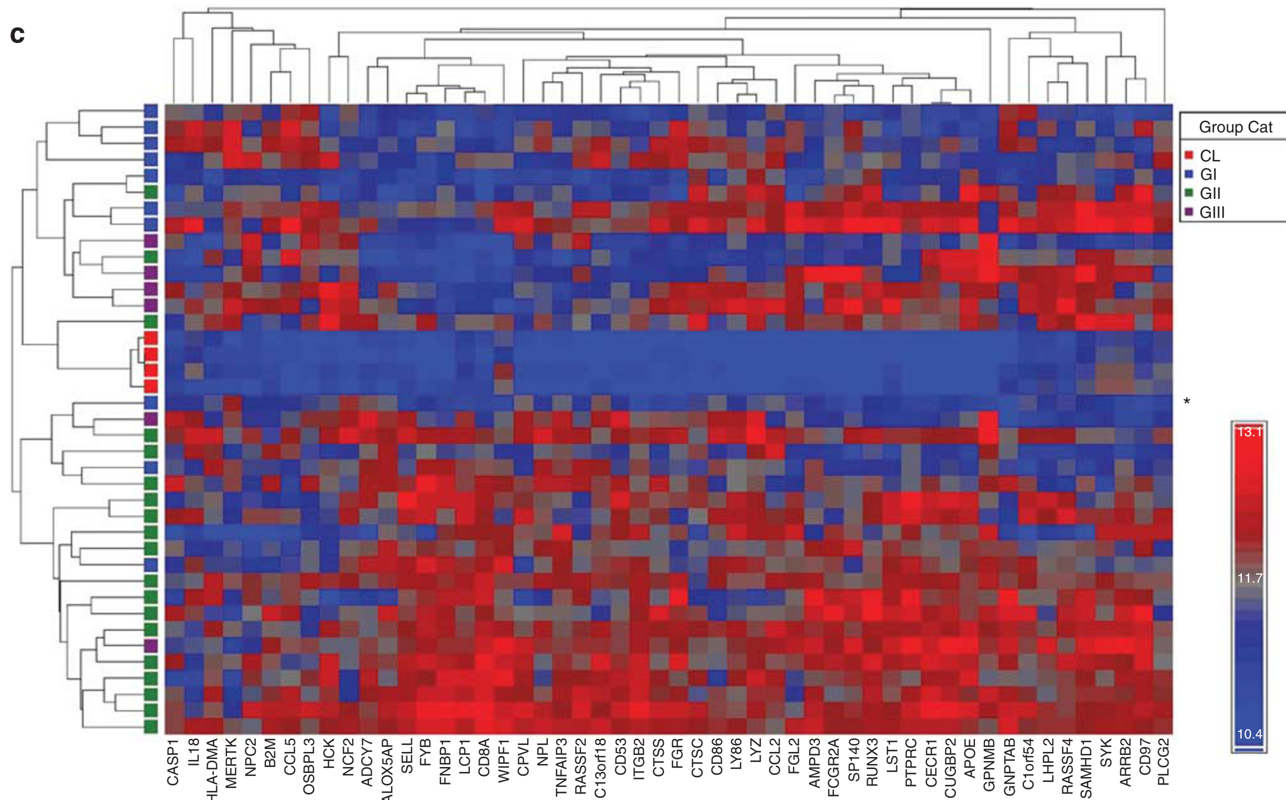
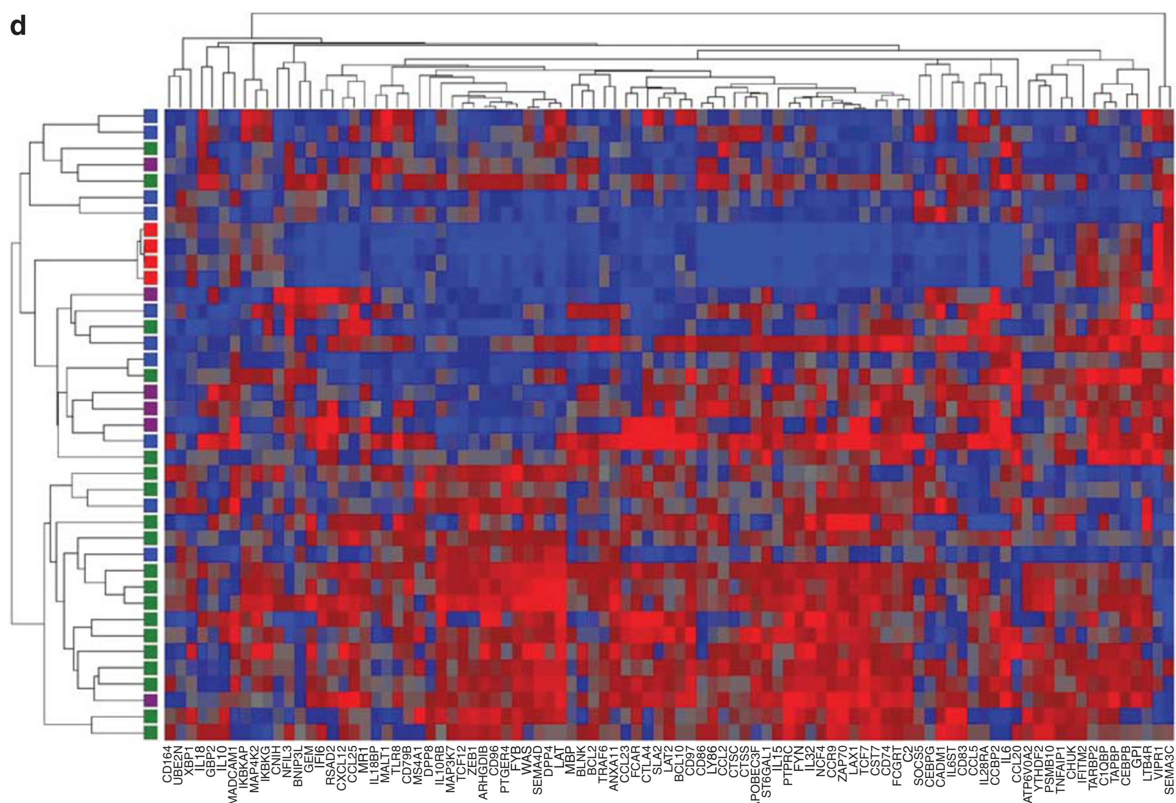


Figure 2 Selected gene sets of differentially regulated genes between IU-TAB-1 cell line and 34 frozen thymomas. (**a**) TE cell surface antigens. (**b**) Stroma-related gene signature, desmoid-type fibromatosis (DTF)-associated genes DTF fibroblast signature. (**c**) Stroma-related gene signature, a macrophage response core gene set-CSF1signature. (**d**) Immune-system-related genes. CL = Cell line, group I (type AB), group II (types B1–B2) and group III (type B3).*Represents the primary tumor where IU-TAB-1 cell line was derived. One duplicate for group 1 was also included for cluster analysis.





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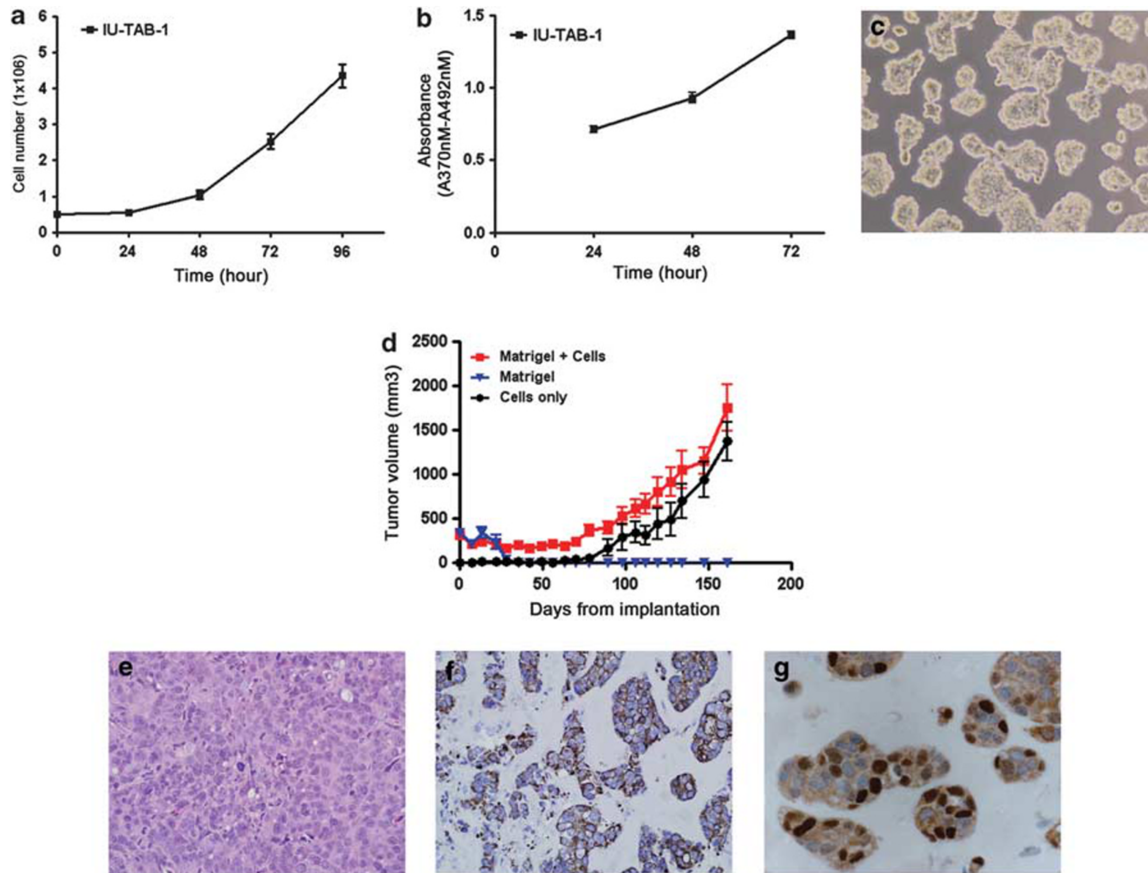

Figure 3 The *in vitro* and *in vivo* functional properties of IU-TAB-1. The growth curve of IU-TAB-1 cell line was shown using (**a**) trypan-blue dye exclusion method and (**b**) BrdU cell proliferation colorimetric ELISA kit (Roche Diagnostics). Data points represent the means of three independent experiments. (**c**) Morphogenesis assay of IU-TAB-1 in Matrigel. (**d**) The tumorigenic potential of IU-TAB-1 cells was assessed by implanting IU-TAB-1 cells into the flanks of NOD/SCID mice with cells alone or mixed with low growth factor-reduced Matrigel (Becton Dickinson, Bedford, MA, USA). (**e** and **f**) Represent the staining of the tumors derived from IU-TAB-1 cells implanted into NOD/SCID mouse. (**e**) Hematoxylin and eosin and (**f**) immunohistochemical staining with positivity for pan-cytokeratin, (high-power magnification $\times 40$). (**g**) Immunohistochemical staining with nuclear positivity for p63 (high-power magnification $\times 40$).

introduced novel targeted therapies in the clinic.[26] However, to date, this has not been the case for thymic malignancies. Mechanistic information regarding the tumorigenesis, progression and recurrence of thymic neoplasms is extremely limited due to the lack of relevant preclinical models. To date, only a few cell lines from tumors of the thymus have been established representing mostly thymic carcinoma.[13–15] In this study, we report a detailed characterization of a novel thymoma cell line (IU-TAB-1), which was derived from a patient with the stage II thymoma (WHO-type AB tumor) and its molecular comparison in regard to the primary thymomas.

Histological and immunophenotype and FACS analyses confirmed the TE nature of this cell line by the presence of epithelial cell markers (pan-cytokeratin and EpCAM/CD326), the presence of cell surface markers of TE cells[18] and nuclear p63,[27] and by the absence of lymphoid and other leukocyte markers as well as T-cell markers.[28–30] We verified that IU-TAB-1 cell line does not express c-KIT (CD117) and EGFR markers, which are typically expressed in thymic carcinomas.[31,32]

Genetic alterations in thymomas have been correlated with the histological WHO subtype and the clinical behavior.[10,21–25,33,34] Using conventional cytogenetics analysis, we identified and correlated chromosomal alterations in the IU-TAB-1 cell line with the reported aberrations. At first glance, there were a surprising large number of aberrations that were identified in IU-TAB-1. This data must be viewed in comparison with data from other benign and malignant cell lines. High frequency of chromosomal aberrations has been detected in cell lines derived from other organ sites. Cell lines derived from 'normal' tissue often show significant number of chromosomal aberrations. For example, MCF10A cell line, a commonly 'normal' breast cell line, harbors multiple chromosome aberrations, some of which might contribute to its immortalized phenotype.[35] In addition, even benign lesions such as fibroadenomas or colonic adenomas harbor chromosomal aberrations,[36,37] albeit at a lower

frequency that malignant tumors. Cytogenetic studies revealed recurrent chromosome alterations in colorectal adenomas.[37] Cell lines established from colorectal adenomas harbor various chromosome aberrations,[38,39] suggesting the involvement of these alterations in the transformation to malignant phenotype. Furthermore, we among others have documented that thymomas are tumors that can behave in a malignant manner. Abnormalites identified in tumors have been attributed to the heterogeneous nature of primary tumors, with the aberrant subset population surviving in the culture system environment. Neve *et al*[26] has reported that the breast cancer cell lines carry more aberrations than primary tumors, although they retained many biological and genomic properties of primary tumors. These studies also support the hypothesis that IU-TAB-1 cell line could provide important knowledge as experimental models for understanding the thymoma biology and testing new drugs.

Our data are consistent with phenotypes described in type AB thymomas reported in earlier studies.[10,21–23,33,34] Chromosome 6q abnormalities, as noted in IU-TAB-1, are among the most frequently seen in thymic neoplasms. Inoue *et al*[21] has reported that all the histological types except A showed multiple recurrent allelic imbalances at chromosome loci besides chromosome 6 aberrations. In addition, our studies showed that all histological types, albeit with different frequencies, can invade and metastasize to extrathoracic sites.[5,6] Also of note, aberrations in chromosome 20p13 (add(20)(p13) were observed in IU-TAB-1. Thymoma insertional mutation-1 transgenic mice, which carry a transgene insertional mutagenesis syntenic to human chromosome 20p13, have been shown to develop thymomas.[40]

Using gene expression profiling based approach, we further analyzed the similarities between the cell line and primary thymomas. IU-TAB-1 retained some of the genes, but clustered separately from primary tumors. These observations can be at least partially explained on the basis of the following: Prior studies on other tumors have also observed that cell lines and primary tumors cluster differently;[41] The absence of tumor microenvironment in the cell line is one of the obvious reasons for the cell line to cluster differently. In this regard, we show that IU-TAB-1 lacks both stromal and immune-system-related genes in contrast to the primary tumor groups, confirming the differences due to the multicellular nature of primary tumors.

The ability of human cancer cell lines to form tumors in immuno-compromised mouse models allows analysis of *in vivo* drug efficacy as preclinical proof-of- concept validation. We have shown that IU-TAB-1 is tumorigenic *in vivo* underlining its potential as a preclinical model for thymic neoplasms. Of note, the slow kinetics of tumor development in the xenograft model of IU-TAB-1 is consistent with its origin in thymoma type AB and goes hand in hand with the features seen in Matrigel morphogenesis assay. These combinatorial results make IU-TAB-1 cell line relevant in the investigation of thymomas.

In conclusion, we report a novel preclinical model for the study of human thymoma that should provide an excellent tool to study the relevance and functional role of novel therapeutic targets in thymomas.

Supplementary Informationaccompanies the paper on the Laboratory Investigation website (http://www.laboratoryinvestigation.org)

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DISCLOSURE/CONFLICT OF INTEREST
The authors declare no conflict of interest.

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