REVIEWS

Discovery tools for solid tumor research

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Abstract

Solid tumors take center stage as a primary human health concern as they comprise the majority of cancer incidences and mortalities. It is heartening however, to note the decreasing incidence rates as well as better survival rates over the past decade, largely owing to preventive measures, healthy life styles and developments in diagnostic, therapeutic and management areas which in turn are due to the rapid progress in cancer research. Many tools are available for cancer research of which, cell lines obtained from either the solid tumor site or from metastatic sites, are pivotal. More than 700 cell lines have been established from solid tumors ranging from the carcinomas and lymphomas to the sarcomas. Over the past decade, developments in cell culture techniques viz. the three dimensional culture systems and xenotransplants, have increased the potential application of such cell lines. Apart from the cell lines and various culture techniques, other material such as tumor tissue biopsies, tissue lysates and tissue arrays from cancerous, adjacent and normal tissue are major contributors to cancer research. While each one has its own advantages and limitations, a combined approach gives us an over-all picture of solid tumor research development over the recent past and the future directions available.

Key words

Solid tumors, Cell lines, Cell cultures, Three D cultures, Xenotransplants, Cancer research

Introduction

A tumor is an abnormal mass of tissue, abnormal in that it contains a large number of cancerous or pre-cancerous cells that have grown in an unregulated manner. Tumors may be classified as solid tumors or leukaemias. Solid tumors are distinguished from leukaemias in that they do not contain any cysts or liquid areas and can arise from any solid organ. Solid tumors also differ from leukaemias in that their development involves a plethora of genetic changes as opposed to say, a single chromosomal translocation in leukemia [1]. Solid tumors are classified according to the source tissue and may be hereditary or sporadic. Sporadic cases may be caused by exposure to certain chemicals such as vinyl chloride or due to radiation exposure [2]. The solid tumor classification, incidences, occurrence modes, diseases that are associated with an increased risk and their predominant associated genetic aberrations are given in Table 1.

In a recent report on cancer status in the American population, the North American Association of Central Cancer Registries (NAACCR) and the National Cancer Institute (NCI) presented a decreasing trend for cancer incidence and a corresponding increase in survival rates [3]. To quote the Director of the National Cancer Institute (NCI), Harold Varmus, "The result of improved methods for preventing, detecting, and treating several types of cancer" and "an obligation to discover and deliver better ways to control all types of cancers" has resulted in the continuing trend for increased survival

rates over the past two decades. Clinical management of solid tumors includes conventional surgical removal coupled with radio/chemotherapy as well as newer management strategies such as the use of small molecule inhibitors and miRNAs.

Cancers of the epithelial and glandular tissue dominate the incidence rates followed by the lymphomas and sarcomas. Lymphomas seem to be more sporadic than hereditary while the sarcomas and carcinomas have both causative aspects. A distinct relation is seen in the increased association between a disease condition to the cancer type and also to the increased genetic aberrations. More than 700 well characterized continuous cell lines have thus far been obtained from human cancers from both the non-metastatic and the metastatic sites. Sarcomas have contributed to more continuous cell lines compared to lymphomas although the incident rates are less than half. All these cell lines which are predominantly attached cell lines are continuing to contribute significantly to cancer research in particular and evolution of cell culture techniques in general.

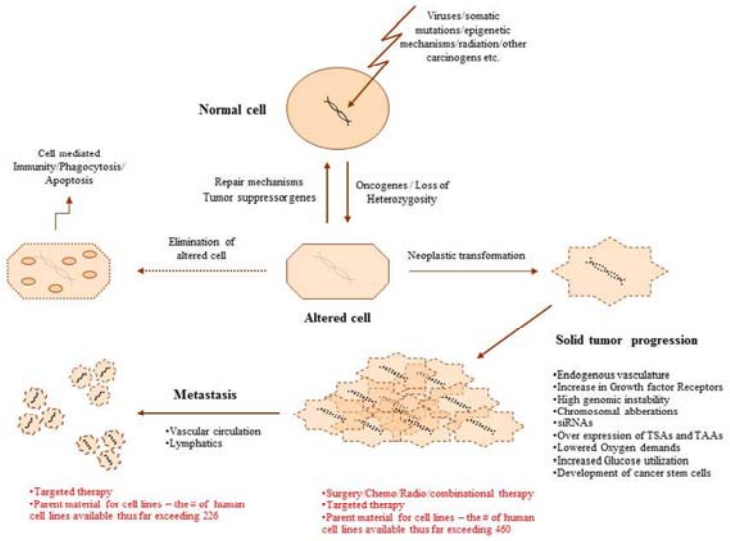
Table 1. Salient features and cells lines from solid tumors

Solid tumor type	Sarcoma	Lymphoma	Carcinoma
% incidence (Population weighted average; as of 2008; WHO estimates)	130,000 (4%)	424,350 (15%)	>2,368,000 (81%)
Type of origin tissue	Connective tissue (muscle, skeletal tissue)	Lymphoid organs (spleen, thymus, lymph nodes)	Epitheloid and glandular organs (including breast, prostate, colon, oesophageal, stomach)
Occurrence mode	Hereditary & sporadic	Sporadic	Hereditary & sporadic
Onset	Adult, childhood	Adult, childhood	Mostly adult
Common associated disease conditions	Retinoblastoma (Soft tissue Sarcoma), Basal cell nevus syndrome (Rhabdomyosarcoma, Fibrosarcoma), Werner syndrome and Li-Fraumeni syndrome	AIDS (Non-Hodgkin's Lymphoma), Sjögren's syndrome (B-cell lymphoma), *Helicobacter pylori* infection	Basal cell nevus syndrome (basal cell carcinoma) Li-Fraumeni syndrome (brain cancer, breast cancer, adrenocortical carcinoma) Familial adenomatous polyposis (Colorectal carcinoma) - Hereditary breast and ovarian cancer (Breast & ovarian cancers)
Common associated cytogenetic abnormalities	Heterozygous germline mutation on one allele and a somatic mutation on the other allele of the *RB1* gene on chromosome 13q14 Somatic mutations in the *PTCH2* gene on chromosome 9q22.3 *TP53* mutation on chromosome 17p13.1 Numerical and structural chromosomal abnormalities leading to loss of function of *WRN* gene on chromosome 8p12	Chromosomal translocations leading to altered *c-myc* expression	Somatic mutations in the *PTCH2* gene on chromosome 9q22.3 *TP53* mutation on chromosome 17p13.1 Mutations in *APC* on chromosome 5q21-q22 Germline mutations in BRCA1 on 17q11 and BRCA2 on 13q12–q13
# of human cell lines derived (NM=Non metastatic site origin, M=Metastatic site origin)*	> 90 (NM) > 20 (M)	< 10 (NM) < 9 (M)	> 361 (NM) > 200 (M)

* data obtained from registered cell line sources such as National Cancer Institute as on submission of this manuscript

The study of solid tumors is important because they comprise more than 95% of all cancer related deaths in any given year. The initial diagnosis and staging of solid tumors is carried out by histopathology. In addition, cytogenetic data from solid tumors is used in diagnosis, targeted therapy, evaluating treatment response of metastatic cancer and as a marker for prognosis in the clinical setting. For tumors in which histologic features overlap, cytogenetics plays an important role for diagnosis, for example in the diagnosis of the t(12; 16) in myxoid liposarcoma, the t(2; 13) in alveolar rhabdomyosarcoma, and the t(X; 18) in synovial sarcoma. The establishment of primary cell cultures from patient biopsies thus continues to play an important role. More than 700 cell lines have been derived from solid tumors as the parent material, from both non-metastatic and metastatic sites. These cell lines have contributed immensely towards cancer research, bringing newer insights into the mechanism of carcinogenesis, complex alterations in gene expression including epigenetic influences, which lead to neoplastic transformation towards the formation of solid tumors. They have also aided in the discovery of several biomarkers, therapeutic targets and management strategies (Figure 1). In this review, we present the important solid tumor cell lines, their culture conditions and their applications apart from touching upon other currently available material for cancer studies. The available credible literature sources including journal publications, on-line product catalogues and specific websites of agencies and results of our own experimental works with solid tumor cell lines were referred to obtain the data and information presented in this review.

Figure 1. Mechanisms of oncogenesis and salient features at each stage



Solid tumor genesis is a multiparametric mechanism involving any one or a combination of several parameters such as somatic mutations, epigenetic changes and exposure to chemical and physical carcinogens. When a normal cell undergoes transformation, the oncogene loss of heterozygosity and suppression of repair mechanisms function together for the initial neoplastic mechanisms. An altered cell can be eliminated at this stage by inherent events such as apoptosis or innate immune mechanisms failing which, further development results in solid tumors progressing into metastatic conditions. The solid tumors and the metastatic cells have provided many continuous cell lines that continue to contribute to cancer research.

Solid tumor cell lines

Culture models

Animal cell culture is a complex process in which cells are grown in a 2 dimensional or 3 dimensional environment under controlled culture conditions. Two dimensional cultures can be grown as either monolayers or suspension cultures. 2D monolayers provide us with a controlled, homogenous and uniform cell population for many applications. With the advent of 3D culture systems, organic and synthetic matrices are being used in an attempt to study cell morphology and complex

cell physiology including cell interactions, migration, invasiveness and aggregate formation, thus bringing them as close to in vivo conditions as possible. 3D cell culture reflects normal cell morphology and promotes better intercellular interactions. What is interesting to note is that the same cell line shows markedly different morphology and physiological characteristics as monolayers and as 3D aggregates.

In 3D cultures, cells form aggregates and grow as spheroids on a suitable matrix. Agarose, Collagen, Fibronectin, Gelatin, Laminin and Vitronectin are the commonly used scaffolds for 3D cultures [4]. 3D cultures employ different methods which include liquid-overlay technique, spinner flasks, roller bottles etc. [5]. Another culture technique that involves the co-culture of different cell types is necessary for studying cellular interactions and has a wide range of other applications from feeder cell provisions to provide specific growth factors such as cytokines, to the study of bystander effects for studies such as radiobiology. Co-culture techniques are also being explored in the area of tissue engineering.

Usefulness of cell cultures for in situ studies

Cell culture has multiple applications for in vitro studies. Monolayers and suspension cell lines are widely used for cell cycle analysis by flowcytometry, in-cell ELISA, cell enumeration and viability assays, assays for apoptosis and necrosis, cell migration assays, proliferation assays such as the clonogenic assay, MTT assay etc. and to study bystander effects in radiobiology. Cell lines also contribute massively towards biomarker discovery. Protein expression and gene expression studies could facilitate the identification of proteins or genes that are differentially expressed in a particular disease. Further, the development of monoclonal antibodies against these proteins or their associated transcription factors could be used in targeted therapy and diagnosis. For example, in A549 cells, GATA 6 antibodies were produced against the transcription factor GATA 6 which has its applications in western blotting, immunofluorescence and immunehisto-chemistry [6].

Usefulness of cell cultures for biomarker discovery

Cancer cells secrete or shed proteins that are potentially useful as biomarkers of the disease. These secreted proteins constitute extracellular matrix proteins, receptors, adhesion molecules and soluble proteins such as cytokines, chemokines, growth factors and proteases. All of these can serve as potential candidates for cancer biomarker discovery thus providing new ways to understand disease mechanisms and facilitate the discovery of novel diagnostic markers and therapeutic targets.

It is important to have efficient model systems for biomarker discovery. Irrespective of the marker being genetic, protein or metabolic, the common feature should be their specificity to a cancer type and usefulness to diagnosis and prognosis owing to its sensitivity. Protein biomarkers of all types including membrane bound, intra cellular, shed or secretory have considerable significance and each type presents with a specific usefulness. For example, membrane bound proteins can be good targets for therapy through monoclonal antibodies.

The challenges for protein biomarker discovery include their low in vivo abundance and subsequent masking by inherent proteins in circulation. Cell cultures thus offer a better model as it is rather feasible to eliminate/minimize the masking proteins in controlled in vitro culture systems. The cell culture systems, by suitable experimental variation of conditions, can be made apt for better expressions of the secretome which can be analyzed for biomarker discovery [7]. In addition, cell lines serve as a simplified model of cancer progression, represent the heterogeneity of cancer and have increased reproducibility. For example, in a study to identify biomarkers for breast cancer, isogenic series of breast cancer cell lines such as non-tumorigenic MCF10A, premalignant & tumorigenic MCF10AT, invasive MCF10 and metastatic MCF 10CA cl. D have been used to discover highly secreted proteins whose abundance changes significantly corresponding with tumor aggressiveness. Using LC-MS/MS proteomic technology, over 250 proteins per cell line were identified and their abundance was represented using a Mascot score. Top ten proteins in each cell line were emphasized largely and observed if these proteins change in abundance across the series of cell lines. From the list, five proteins were selected among which serpin peptidase inhibitor (AACT; alpha-1-antichymotrypsin; SERPINA3), serine (or cysteine) proteinase inhibitor, clade

A (AAT; alpha-1-antitrypsin; SERPINA1), galectin-3-binding protein (LGALS3BP; lectin, galactosidebinding, soluble, 3 binding protein; 90K) and secreted protein, acidic, rich in cysteine (SPARC; osteonectin) were seen to be more highly secreted from the tumorigenic cell lines while mesothelin isoform 1 (MSLN) was expressed more in the non-tumorigenic and non-metastatic cell lines. The differential expression of proteins was validated using western blot analysis [8]. Such experimental designs that exploit large scale secreted protein profiles to distinguish between aggressive and non-aggressive cancer phenotypes, point out to the promise of using cell lines as models for biomarker discovery.

Using a protein chip platform to analyze plasma for biomarker discovery is highly challenging due to the presence of highly abundant serum proteins which mask proteins of low abundance that could otherwise be potential tumor markers. Using the SELDI protein chip platform, 39 human cancer cell lines were therefore analyzed and a 12 kDa protein biomarker candidate was identified in colon cancer cells. This protein was found to be Prothymosin-α by ProteinChip time-of-flight mass spectrometry and ProteinChip-Tandem MS systems [9]. This study thus reiterates the potential of cell line secretomes for biomarker discovery using a protein chip platform.

In another study, a bottom-up proteomics approach was performed using two-dimensional LC-MS/MS systems in four lung cancer cell lines- H23 (adenocarcinoma), H520 (squamous cell carcinoma), H460 (large cell carcinoma) and H1688 (small cell lung cancer). The conditioned media from cultures of these cell lines was analyzed to identify secreted or shed membrane-bound proteins that could be useful as novel lung cancer biomarkers. A total of 1,830 proteins were identified of which five candidates- ADAM- 17, osteoprotegerin, pentraxin 3, follistatin, and tumor necrosis factor receptor superfamily member 1A- were validated in the serum of normal and diseased individuals [10].

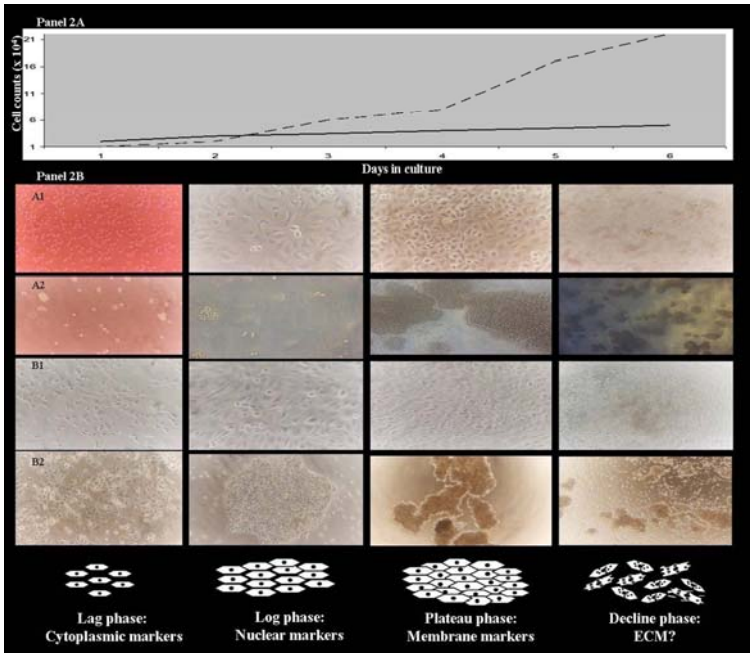
A similar study was performed on the ovarian cancer cell lines HTB75, TOV-112D, TOV-21G and RMUG-S in order to identify novel biomarkers using proteomics and mass spectrometry. The conditioned media was analyzed by two dimensional liquid chromatography-mass spectrometry. A total of 2,039 proteins were identified of which, apart from the already known markers, a list of 51 potential candidates was generated. From this 10 proteins were selected for validation in serum. Clusterin, a glycoprotein which seems to be an apoptosis inhibitor, showed a significant difference in expression in the serum of normal individuals and ovarian cancer patients [11]. Another protein, NPC2, was found to be highly expressed in ovarian cancer tissue but not in sera on immunohistochemistry analysis [12].

Limitations of traditional cell culture models

While conventional 2D culture systems are powerful tools to understand how cells proliferate, grow and respond to stress, 2D plastic substrates are nevertheless considerably limited in reproducing the complex 3D environments existing in vivo. In addition, cells grown on 2D plastic substrates are forced to adapt to an artificial, flat, rigid surface, resulting to some degree of misrepresentation of findings, including altered metabolism and declined functionality. Extensive studies have shown that growing cells within 3D scaffolds diminishes the gap between cell cultures and physiological tissues. Therefore, a 3D cell culture system may prove to be of a tremendous advantage over conventional 2D cell culture system [13].

In our own studies using simple agarose gels as a 3D matrix, we obtained aggregates of solid tumor cell lines. Such aggregates were seen to behave fundamentally different from their 2D monolayer counter parts and also to exhibit unique cell line specific features in morphology, physiology and other cellular and aggregate characteristics. It was our observation that survival as aggregates is markedly higher when compared to the monolayers. This is as a result of reduced doubling time, better survival for longer culture durations and an overall ‘expanded’ lag, log, plateau and decline phases in the 3D systems. It was also observed that cells at a particular culture phase were more suited for identifying specific localized biomarkers. When proteins at various culture phases were isolated from cells grown in 2D and 3D systems and analyzed, it was found that the lag, log, plateau and decline phases were better suited for the study of cytoplasmic, nuclear, membrane bound and secretory/extracellular proteins respectively (Figure 2).

Figure 2. 2D and 3D solid tumor cell culture systems for cancer research



Cancer cell lines obtained from solid tumors have contributed in several ways towards cancer research including cell behaviour, biomarkers and drug discovery. The 2D monolayer culture systems have inherent advantages and applications. The 3D culture systems provide aggregates that show distinct morphological features based on the cancer type and culture conditions, demonstrating a much slower progression through the culture stages such as lag, log, plateau and decline phases when compared to monolayers. Panel 2A shows the cell counts for the lung Adenocarcinoma cell line NCI-H23 in 2D (dotted line) and 3D cultures (solid line) in 24 hour intervals indicating the fundamental differences that such systems present. Panel 2B shows the differences in culture morphology of 2 cell lines, A549 (A1 and A2) and NCI-H23 (B1 and B2) through the various culture phases as monolayers and as 3D aggregates respectively. Although both cell types are originally from lung Adenocarcinoma, the culture morphologies as 3D aggregates are strikingly different for the two cell types. While A549 forms aggregates that are lodged on the agarose matrix surface, the NCI-H23 forms floating aggregates with loosely packed cells. Apart from the advantages that such morphological differences offer, we also found that cells at a particular stage of the culture phase are ideal for studies pertaining to specific localized protein expressions. Additionally, culture matrices and spent media of 3D cultures beyond the decline stages might have importance for secretory/extracellular matrix biomarker discovery.

3D co-culture systems could also provide a useful tool in biomarker discovery. Whereas in 2D cultures, the interaction of cells is of the same type, when cells are co-cultured, a heterotypic environment is created thereby allowing true tissue-like interaction. In this type of culture, tumor cells when co-cultured with stromal cells can mimic the actual tumor microenvironment. This thus holds much promise in cancer biomarker discovery.

Xenografts

Along with continuous cell lines, xenografts in murine models have important roles in cancer research. For example, certain solid tumor cell lines can be maintained continuously by passaging in murine models. Xenografts can be developed either by transplanting a piece of human tumor tissue into the mice or by injecting the tumor cell suspension prepared from the tissue biopsy. Athymic nude or immuno deficient mice prove to be useful recipients for grafts and protocols have been well established for obtaining human solid tumor xenograft tissue [14]. Such xenografts are utilized for studies involving receptor mediated growth inhibitory mechanisms [15], effects of hypoxia on differential gene expressions [16] for drug efficacy and resistance studies [17] for clinical trials for a variety of therapeutics and studies involving cancer stem cells.

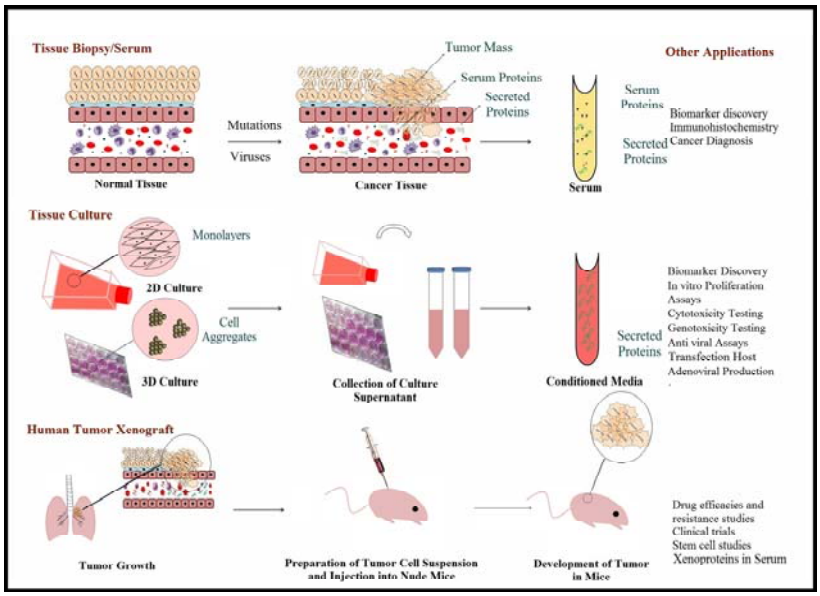
Some xenograft models have also been used to study the bystander effects of radiotherapy in Rhabdomyosarcoma [18] and also to assess the time dependent angiogenic changes in the pathogenesis of Hepatocellular carcinoma [19]. Another interesting study established intracranial brain tumor xenografts in mice, monitored tumor growth and observed the effects of treatment on tumor size reduction by Bioluminescence imaging [20].

Alhough much cancer research involves experiments using xenograft models, they have their limitations because only one cell phenotype is assessed whereas a tumor usually exists of a large array of cellular phenotypes. Xenograft models also create an altered interaction between the tumor and its surrounding microenvironment [19]. Although, mouse models have constituted the major preclinical screen for the development of novel cancer therapeutics, many of them have ended up failing at the stage of clinical trials [21].

Material other than continuous cell lines for cancer research

A number of commercial enterprises now make available, human tumor tissue and normal tissue sourced from institutions under strict IRB and ethical consenting practices. OriGene (USA and China) provides normal and tumor tissue sections, tissue microarrays, tumor tissue protein lysates and whole tissue DNA/RNA. BioChain (USA) provides tissue arrays, tissue sections and protein lysates, Ontario tumor bank (Canada) is a supplier of tissue sections, ANSA tumor tissue repository (India) and Triesta sciences (India) are suppliers of brain tumor tissue and breast cancer and colorectal cancer samples respectively. Apart from these, several organizations and suppliers provide well characterized cancerous core, surrounding and matched non-cancerous tissues, arrays and lysates. Usually provided as paraffin embedded or frozen tissue sections, each tissue source block includes an abstracted pathology report, information on the tissue of origin/site of finding, disease staging, a digital H&E image and the donor's basic demographic information. Some establishments even go one step further in providing matched cancer and normal samples, sometimes even from the same donor. In addition, these commercial enterprises also provide other related services for cancer research such as tissue cDNA arrays and multiplex histologic analysis. Tissue cDNA arrays are useful for differential gene expression profiling, tumor protein lysates have important applications in differential protein expression profiling and biomarker discovery and multiplex histological analysis can be performed by such commercially available tissue microarrays. A comprehensive list of commercial and non-commercial biorepositories and tissue banks globally is also available online at Specimencentral.com.

Figure 3. Material sources for solid tumor research



Solid tumor research is aided by several materials available of which tissue biopsies, cell cultures *in vitro* and xenografts play important roles. Each of them has their own unique applications, advantages and limitations. A combined approach with techniques that mimic *in vivo* conditions to the closest extent, have the best potential for translating research output into patient benefits.

The advantages of these comprehensive tissue products is thus immediately apparent in that it provides researchers with ready-to-use tools for studies in biomarker discovery and validation, cancer drug target identification and validation, diagnostic assay development, and personalized medicine for cancer patients. This commercially available material can be used to correlate the histopathological characteristics of a tumor tissue with normal tissue even with very small amounts of samples.

The disadvantages of using these commercial products however is in that there will be sample variation and this would affect and make very difficult, the study of a generic pathway leading to tumor progression or establishing a standard assay for identification and study of a tumor type. Additionally, the current storage protocols may alter the characteristics of the tissue and hence lead to false positive/negative results. Also such individually sourced samples might have limitations for retrospective studies.

Conclusion

Solid tumor diagnosis, therapeutics and management have seen a major change over the past decade and are probably one of the fastest developing areas of human health care and research globally. Different types of material such as tumor biopsies, cell lines and xenotransplants are currently available and each has a distinct role to play towards cancer research (Figure 3).

The constant technological improvements in utilizing these materials such as better in vitro models, assay methods, efficient drug and biomarker discovery approaches are leading to better understanding of the solid tumor physiology and also better research output translational benefits towards patient health care.

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Conflict of interest

The author declares that there is no conflict of interest statement.

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