

# Cell Lines as In Vitro Models for Drug Screening and Toxicity Studies

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**ABSTRACT** Cell culture is highly desirable, as it provides systems for ready, direct access and evaluation of tissues. The use of tissue culture is a valuable tool to study problems of clinical relevance, especially those related to diseases, screening, and studies of cell toxicity mechanisms. Ready access to the cells provides the possibility for easy studies of cellular mechanisms that may suggest new potential drug targets and, in the case of pathological-derived tissue, it has an interesting application in the evaluation of therapeutic agents that potentially may treat the dysfunction. However, special considerations must be addressed to establish stable in vitro function. In primary culture, these factors are primarily linked to greater demands of tissue to adequately survive and develop differentiated conditions in vitro. Additional requirements include the use of special substrates (collagen, laminin, extracellular matrix preparations, etc.), growth factors and soluble media supplements, some of which can be quite complex in their composition. These demands, along with difficulties in obtaining adequate tissue amounts, have prompted interest in developing immortalized cell lines which can provide unlimited tissue amounts. However, cell lines tend to exhibit problems in stability and/or viability, though they serve as a feasible alternative, especially regarding new potential applications in cell transplant therapy. In this regard, stem cells may also be a source for the generation of various cell types in vitro. This review will address aspects of cell culture system application, with focus on immortalized cell lines, in studying cell function and dysfunction with the primary aim being to identify cell targets for drug screening.

**KEYWORDS** Cell line, Drug screening, Toxicity, Neuronal, Muscle, Myocardium

## CELL CULTURE: IN THE BEGINNING

Cell culture can be traced back to Sir William Harvey in the 16th century, who observed that a piece of myocardium kept in the palm of his hand covered in his own saliva could remain contractile for extended periods of time (Harvey, 2001). Several important landmarks in the development of culture techniques are identifiable since these initial observations. In this regard, Roux, in the late 19th century (Rensberger, 1998), showed that embryonic chick cells can be maintained alive in a saline solution outside the animal body. Later, Harrison showed that amphibian spinal cord

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could be cultured in a lymph clot, demonstrating that axons are produced as extensions of single nerve cells (Harrison, 1910).

Nevertheless, the principle of time limit in such tissue preparation soon became evident, as cells eventually underwent senescence and death. The first indications of cell transformation come from Rous in 1910, who successfully induced a tumor by using a filtered extract of chicken tumor cells, later shown to contain an RNA virus (Rous sarcoma virus) (Rous, 1910). This landmark discovery prompts the notion of *in vitro* immortalization, and demonstrates the concept of enhanced tissue survival in culture, and therefore, the possibility of producing cell models. By the middle of the 20th century, Earle and colleagues isolated single cells of the L cell line and showed that they form clones of cells in tissue culture (Earle, 1943). Later, Gey and colleagues established probably the most well known *continuous* line of cells derived from a human cervical carcinoma, which later become the well-known HeLa cell line (Jones et al., 1971).

However, concerns began to arise regarding loss of function with such immortalization processes. Hence, researchers in this field faced the challenge of preserving or rescuing differentiated traits in the cell lines generated using externally applied stimuli such as media supplementation or modifications, and even the use of physical agents. In this regard, the pioneering work of Rita Levi-Montalcini in 1954, who observed that nerve growth factor (NGF) stimulates the growth of axons in tissue culture, called attention to the immediate environment of cultured cells (Cohen & Levi-Montalcini, 1957; Levi-Montalcini & Cohen, 1960). Later, Eagle made the first systematic investigation of essential nutritional requirements of cultured cells and found that animal cells propagate in a defined mixture of small molecules supplemented with serum proteins (Levintow & Eagle, 1961). Later, Littlefield introduced HAT medium for selective growth of somatic cell hybrids. Together with the technique of cell fusion, this made somatic-cell genetics accessible (Littlefield, 1964). By 1965, Ham had introduced a defined, serum-free medium that could support the clonal growth of certain mammalian cells (Ham, 1965).

The first immortalized cell line to retain differentiated traits of the originating tissue were described in 1968, when Augusti-Tocco and Sato adapted a mouse

nerve cell tumor (neuroblastoma) to tissue culture and isolated electrically excitable clones that extend nerve processes (Augusti-Tocco & Sato, 1969). A number of other differentiated cell lines were isolated at about the same time including skeletal muscle and liver cell lines. Later, Sato himself and other associates published the first of a series of papers showing that different cell lines require differing mixtures of hormones and growth factors to grow in serum-free medium (Clark et al., 1972; Posner et al., 1970, 1971; Sato et al., 1970, 1971).

The isolation and culture of pluripotent embryonic stem cells from mice is also an important landmark (Evans & Kaufman, 1981; Martin, 1981), initiating a field which to this day enables the exciting notion of a cell type that can potentially generate any given tissue if stimulated appropriately. This was followed in 1998 by the groups headed by Gearhart (Thomson et al., 1998) and Thomson (Shamblott et al., 1998), who successfully isolated human embryonic stem cells.

The number of cell lines currently in existence is vast. The American Type Culture Collection currently holds over 4,000 cell lines from over 150 different species, and significantly more probably exist in Patent Repositories throughout the world. The vast scope of different tissue origins and species precludes broad based review of all cell lines. Hence, we have chosen to narrow down this review to three origins of particular interest: skeletal and cardiac muscle, and neuronal tissue. This review provides an insight of the possible use of cell lines from the aforementioned origins as drug screening models that are readily accessible and can potentially prove useful for drug development.

## MYOCARDIAL CELLS IN CULTURE

The use of myocardial cells in culture as a model for the study of the physiology of heart cells increased dramatically by the 1970s (Jacobson & Piper, 1986; Wollenberger, 1985). In order to circumvent the problems posed by the heterogeneity of cells in heart tissue (no more than 20% are myocytes) (Jacobson & Piper, 1986), several procedures for heart myocyte culture have been developed. Most of the early studies were performed in primary cultures of embryonal or newborn tissues, but methods for cell cultures from

adult animals were also developed. These studies refer to primary cultures which can be maintained for weeks and even up to several months in vitro but will inevitably stop growing and die. A myocardial cell line in permanent culture that displays markers of differentiated heart cell function would represent an important advantage over primary cultures, and it could also provide large amounts of homogeneous cell populations to many laboratories throughout the world to study such particular markers. Techniques used to immortalize cardiac myocytes have involved fusion with transformed cells in order to establish functional cardiocyte clones and transformation of primary myocardiocytes by tumor viruses or by carcinogens, none of which have been fully successful (Wollenberger, 1985). A clonal muscle cell line, probably derived by “spontaneous transformation” of BDIX rat embryonic enriched cardioblasts, has been described (Kimes & Brandt, 1976) but not extensively studied (Muller et al., 1986). Cells obtained by transfection with large T antigen gene of the SV-40 oncogenic virus, a quite classical cell transformation protocol (Sen et al., 1988), or from atrial tumors developed in transgenic mice (Behringer et al., 1988; Steinhilper et al., 1990) are either not immortalized, or they proliferate for only a limited number of passages (Behringer et al., 1988; Delcarpio et al., 1991; Sen et al., 1988; Steinhilper et al., 1990), or give rise to clonal cell lines that lack differentiated markers (Behringer et al., 1988). Cell lines obtained from human (Wang et al., 1991) and avian (Jaffredo et al., 1991) transfected embryonic heart cells as well as the old H9c2 embryonic cardiac cell line [a subclone of that established by Kimes and Brandt (1976)], and reinvestigated by Hescheler et al. (1991) and Sipido and Marbán (1991) have been reported to maintain some differentiated markers of cardiac cells in culture. Other efforts have yielded immortalized cell lines by spontaneous transformation, such as the MHEC5 cell line (Plendl et al., 1995), but this particular tissue is endothelial in nature. Further, stem cell derived myocardial cells exhibit a strong myocardial phenotype, but they undergo terminal differentiation and/or eventually die (Boheler et al., 2002; Pasumarthi & Field, 2002). Efforts to circumvent this issue have involved gene transfer strategies, with positive effects in stimulating the cell cycle. Briefly, Huh et al. (2001) determined that the abrogation of p53 by its

interaction with the SV-40 T antigen binding protein, is important in preventing death of embryonic stem (ES) cell-derived cardiomyocytes. Elegantly, this group transfected a mutant SV-40 T protein that lacked the p53 binding domain in such cells, documenting widespread cell death with apoptotic features. In another interesting article, Pasumarthi et al. (2001), demonstrated expression of adenoviral E1A in cardiomyocytes, which results in the activation of DNA synthesis followed by apoptosis, can be reverted by coexpression of prosurvival mutant forms of p53 and p193 (a member of the BH3-only proapoptosis subfamily). These results combined explain the phenotypic differences of E1A versus T-antigen gene transfer in cardiomyocytes, and how several different pathways may be involved in rescuing cardiac cells from death in vitro.

Another approach to obtaining in vitro cardiomyocyte models has involved the use of multipotent cell lines, such as the P19CL6 embryonal carcinoma (Young et al., 2004), which can be induced to differentiate to beating cardiomyocyte-like cells when cultured with DMSO, a rather non-physiological stimuli. However, only confluent cultures (>70%) are able to differentiate in such conditions, and it has not been possible to measure this efficiency on a cell-by-cell basis (Peng et al., 2002), and hence the possibility of contaminating cell types cannot be excluded. In fact, preliminary electron-microscopic studies suggest the presence of cells other than cardiac myocytes (Peng et al., 2002). About five years ago, several groups suggested that bone marrow stromal (BMS) cells display several characteristics of a pluripotent mesenchymal stem cell. Such BMS cells, for example, can differentiate into multiple cell lineages, including bone, muscle, and fat. Makino et al. (1999) hypothesized that these cells have the potential to differentiate into cardiomyocyte precursors. Indeed, after immortalizing BMS cells by prolonged culture in vitro, they were able to identify a single clone of adherent fibroblast-like cells that, when treated with 5-azacytidine, reproducibly differentiate into beating myocytes displaying many properties of embryonic ventricular muscle. After differentiation, these cells, termed CMG (cardiomyogenic), acquired many morphologic features of cardiac muscle, including sarcomeres, one to three centrally located nuclei, and atrial granules (which are also seen

in embryonic ventricular cardiomyocytes). They also expressed several cardiac-specific genes, including the GATA4 and Nkx2.5 transcription factors and the brain natriuretic peptide (BNP) and atrial natriuretic factor (ANF) genes. In addition, they displayed cardiac-like action potentials with a shallow resting membrane potential, a long action potential duration, and a late diastolic slow depolarization current. However, they display both sinus node-like or ventricular myocyte-like action potentials, suggesting the presence of multiple cell types.

From the above, one can infer that the lack of in vitro models of cardiac function has been overcome to some extent, but inherent problems are present in currently existing models. The need to use non-physiological stimuli, and the production of several cell types in culture “contaminate” the models by introducing other variables, limiting the potential use of such models in drug screening. A permanently growing cell line that differentiates into one cell type is then quite desirable, and could be further “purified” by cloning. In this regard, the RCVC cell line (Caviedes et al., 1993) appears as an interesting possibility. This line was established by a proprietary immortalization protocol, named UCHT1, which induces permanent immortalization in vitro, yielding cell lines that exhibit differentiating traits. Briefly, this protocol utilizes the pro-immortalizing activity of soluble factors produced by the UCHT1 cell line, which induces immortalization of tissues established in primary culture conditions after variable periods of sustained exposure. In this regard, RCVC cells, after differentiation with media supplemented by soluble, physiological factors such as hormones and trace elements, express muscle markers by immunohistochemistry (myoglobin,  $\alpha$ -sarcomeric actin,  $\alpha$ -actinin, and desmin) and develop voltage gated  $\text{Ca}^{2+}$  currents that are characteristic of mature cardiomyocytes (ascertained by  $^3\text{H}$  nitrendipine binding,  $^{45}\text{Ca}^{2+}$  influx, and patch-clamp electrophysiology), after periods of up to one month of differentiation with media supplemented by soluble factors such as hormones and trace elements, and serum reduction (Caviedes et al., 1993). The stable expression of these traits, and again, the possibility of cloning to generate homogeneous tissue may prove very important for the pharmaceutical industry, particularly as a bioassay to test therapeutic and toxic effects of drugs that

modulate  $\text{Ca}^{2+}$  channel activity in the cardiovascular domain (i.e., anti-hypertensive compounds).

## SKELETAL MUSCLE

The use of cell lines to develop in vitro models of muscular function has been desirable for quite some time, but due to the wide range of processes that contribute to mechanical activation in skeletal muscle, functional models are limited. Indeed, early events in excitation–contraction coupling involve several ion channels and receptors, namely nicotinic acetylcholine receptors at the neuromuscular junction, voltage dependent sodium and potassium channels at the sarcolemma and T-tubule membranes, voltage dependent dihydropyridine receptors in the T-tubule, and one or more types of calcium release channel in the sarcoplasmic reticulum. On the other hand, voltage dependent calcium release may be related to mechanisms other than excitation–contraction coupling, i.e., processes related to myogenesis itself. Expression of various ion channels have been studied both in rat embryo and in developing muscle cells in vitro:

1. Dihydropyridine receptor density increases sharply upon development (Kyselovic et al., 1994; Romey et al., 1989);
2. L-type calcium currents parallel the onset of dihydropyridine receptor expression (Cognard et al., 1993; Kidokoro, 1975; Romey et al., 1989; Shimahara & Bournaud, 1991);
3. T-type calcium currents appear transiently during muscle development (Gonoi & Hasegawa, 1988; Rohwedel et al., 1994) but not during primary culture differentiation (Cognard et al., 1993);
4. Sodium channels and sodium currents also increase during muscle development (Frelin et al., 1983; Gonoi et al., 1985; Sherman et al., 1983; Weiss & Horn, 1986). In mammalian muscle, large shifts in tetrodotoxin (TTX) sensitivity occur during muscle development both in vivo and in vitro and the simultaneous use of ion flux and radioligand techniques has led to the definition of several sodium channel subtypes in developing muscle cells in culture (Frelin et al., 1983, 1984);
5. Potassium channel genes seem also to be developmentally regulated both in embryonic muscle and in a muscle cell line (Lesage et al., 1992);

6. Acetylcholine receptor channels also change during muscle development (Mishina et al., 1986; New & Mudge, 1986); and
7. Ryanodine receptor channels are seen only in mature muscle cells (Airey et al., 1991; Kyselovic et al., 1994; Marks et al., 1989). Of particular interest are the temporal differences in the induction of dihydropyridine receptors and ryanodine receptors during skeletal muscle development (Kyselovic et al., 1994), results that suggest that coupling between excitation and calcium release may differ in different developmental stages.

Hence, cell lines that reproduced myogenesis stably are most desirable. Regarding the establishment of skeletal muscle cell lines, one notable example is the mouse-derived myoblast C2C12 line, established by Yaffe and Saxel (1977a, 1977b). This cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. However, one important caveat of C2C12 cells is that they require intense surveillance, as they undergo terminal differentiation upon reaching confluence, hence depleting the myoblast stock. Other models, such as the L6 rat-derived myoblast line, was isolated originally by Yaffe from primary cultures of rat thigh muscle maintained for the first two passages in the presence of methyl cholanthrene (Yaffe, 1968). L6 cells fuse in culture to form multinucleated myotubes and striated fibers. However, the extent of cell fusion declines with passage and the cells must be frozen at low passage and periodically recloned with selection for fusion competent cells.

The major drawbacks with currently existing skeletal muscle models essentially deal with general cell culture issues, such as senescence or loss of function with time, and the possibility of terminal differentiation and the consequential loss of the myoblast population. In this regard, one cell line of human origin, the RCMH cell line (Caviedes et al., 1992) exhibits muscle specific markers, expresses functional muscle-specific ionic channels (Liberona et al., 1997) inositol 1,4,5-trisphosphate (IP3) receptors (Liberona et al., 1998), and can fuse to form multinucleated structures. These cell lines could provide valuable tools to the pharmaceutical industry, as they possess several attractive targets that modulate

skeletal muscle contraction (i.e., ion channels, IP3 receptors). This could apply to conditions where muscle relaxation is desirable (surgery, mechanical ventilation, etc.).

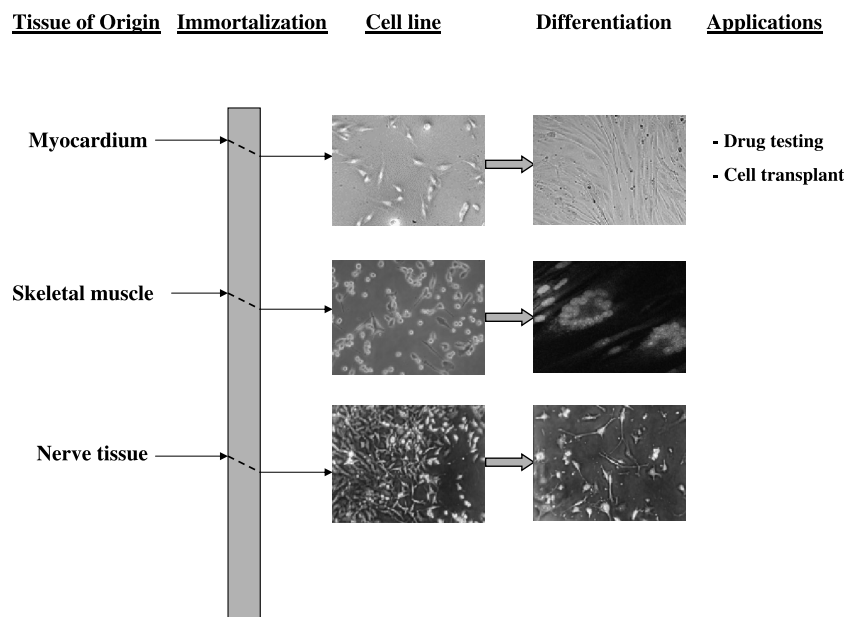
Interestingly, a muscle cell line derived from a patient bearing Duchenne's muscular dystrophy, the RCDMD cell line, expresses altered voltage activated calcium currents (Caviedes et al., 1994) and increased IP3 mass and receptors (Liberona et al., 1998). The importance of such pathological-derived permanent models is evident, and is comparable to that of cell line derived from normal tissues. The former offers the unique possibility of studying cell pathophysiology, and the identification of anomalous functions that could be targeted pharmaceutically.

## NEURONAL TISSUE

The central nervous system is regarded as the final frontier in biomedical research. Its complexity is certainly reflected in the high order of functions for which it is responsible. Hence, the complexities and diversity of pathology based in such tissues is vast and range from loss of motor function to behavioral and intellectual dysfunction. Further, neurodegenerative diseases are an even more critical public health issue considering the rapidly growing elderly population. Further, genetic diseases that compromise neuronal function remain a major therapeutic challenge. The central nervous system is then an enormous target for drug development, and models are clearly warranted.

Ethical and practical problems present clear ethical and moral issues in procurement and use of human tissues to carry out cellular pathophysiological studies in any given genetic alteration. Thus, animal models have been established for a number of such diseases, in particular the use of genetically-modified animal models (knockout, transgenics). However, many of such models have limitations in the quantity of obtainable tissue; the fact that many such models result in premature death or still birth is a significant concern. Further, there is also the issue that compensatory mechanisms in the case of non-lethal manipulations are resultant, which may mask the effects of the human disease to study.

As previously mentioned, since the discovery of neuroblastoma cell lines in the 1960s, the notion of



**FIGURE 1** Schematic Representation of the Process to Generate Cell Lines. Tissues Placed in Culture from the Origins Noted Can Be Immortalized or Transformed By Various Procedures (i.e., Viral Transfection of Oncogenes, Exposure to Carcinogens, etc.). Cell Lines Generated May or May Not Retain Differentiated Traits, but Morphological Changes are Evident, and Enhanced Proliferation is Attained. If Necessary, the Cell Lines May be Submitted to External Manipulations to Induce or Enhance the Expression of Differentiated Traits, Generally Accompanied by a Substantial Reduction in Proliferation or Arrest. Possible Applications of the Cell Lines in Differentiated States Include Drug Testing (i.e., Adrenergic Drugs in Myocardial Cell Lines, Cholinergic Compounds in Skeletal Muscle Lines, Neurotransmitter Agonists in Nerve Cell lines). Micrographs of Cell Lines Correspond to: Myocardium, H92c [©Society for Endocrinology (2002), Taken from van der Putten et al. (2002). Reproduced by Permission]. Skeletal Muscle, RCMH. Nerve Tissue, RCSN.

immortalized neuronal cell lines has become feasible. Since then, several other such cell lines have become available, including an example derived from human tissue, such as the NT2 cell line (Andrews et al., 1984; Borlongan et al., 1998; Flax et al., 1998). This cell line is derived from a lung metastasis of a 22-year-old Caucasian male with a testicular germ cell tumor. This line, when cultured in retinoic acid, and then exposed to mitotic inhibitors for 2 weeks, yields a typical culture that contains approximately 10–20% neurons, commonly referred to as hNT neurons (Andrews, 1984).

Nevertheless, the problem of stability, variability and quantity of cells, in vitro senescence along with de-differentiation, and subsequent loss of function are still a major issue. This is particularly pertinent to conditions of a clear genetic origin. One clear example is the trisomy 16 mouse, an animal model of human trisomy 21 (Down's syndrome) (Cox & Epstein, 1985; Cox et al., 1980, 1981). Because this condition presents as non-viable in the mouse, and considering the inherent limitations of primary culture techniques, the establishment of immortalized cell lines appears as an excellent alternative to preserve the trisomic

condition indefinitely in vitro, and thus generate immortal models that are clonable, easy to access and manipulate, and that provide unlimited amounts of cells. In this regard, the first attempts to immortalize trisomy 16 mouse tissue using oncogene transfection methods were unsuccessful (Frederiksen et al., 1996; Kim & Hammond, 1995). These were due fundamentally to viability problems or poor stability of neuronal traits after transformation. As previously described, our laboratory has used an original procedure (Caviedes et al., 1993), which has resulted in a successfully established neuronal cell line in permanent culture from diverse origins of the CNS of normal and trisomic mice. These cultured lines have been demonstrated to retain neuronal traits and be stable after eight years (Caviedes et al., 1993; Liberona et al., 1997). These efforts were the first to show establishment of continuously growing cell lines expressing stable neuronal phenotypes and lacking glial features. Our experience with these and other cell lines comparably demonstrates that, with this procedure, the lines generated retain differentiated traits, some for decades (Allen et al., 2000; Cárdenas et al., 1999; Caviedes et al., 1993; Liberona et al., 1997). In

**TABLE 1** Brief Description of Some Currently Available Cell Lines from Myocardial Skeletal Muscle and Neuronal Origin

Cell lines	Tissue	Immortalization	Features	References
H9c2	Subclone of BDIX embryonic rat heart cell line	Spontaneous	Skeletal muscle properties, fusion, response to acetylcholine stimulation.	Hescheler et al., 1991;
MHEC5	Mouse heart	Spontaneous, malignant	Endothelial (hemangioendothelioma), tumorigenic. Expresses vascular cell adhesion molecule-1	Kimes and Brandt, 1976; Sipido and Marban, 1991; Plendl et al., 1995
CMG	Mouse bone marrow stroma	Spontaneous	Forms myotube-like structures, sarcomeres, beating after two weeks. Expression of atrial natriuretic peptide, brain natriuretic peptide, myosin, desmin and actinin.	Makino et al., 1999
RCVC	Adult rat heart muscle	UCHT1 protocol	Muscle markers (myoglobin, $\alpha$ -sarcomeric actin, $\alpha$ -actinin and desmin), mature $\text{Ca}^{2+}$ channels.	Caviedes et al., 1993
C2C12	Mouse, skeletal muscle	Spontaneous, after serial passaging	Fusion into terminal mature, contractile myotubes. Expression of ryanodine receptors.	Yaffe, 1968; Yaffe and Saxel, 1977a, 1977b
RCMH	Adult human, skeletal muscle	UCHT1 protocol	Expression of muscle specific markers, functional muscle-specific ionic channels, and IP3 receptors. Fusion, forming multinucleated structures.	Caviedes et al., 1992; Liberona et al., 1997, 1998
Neuro blastoma (several)	Mouse brain neuroblastoma C1300	Serial passage	Morphology of mature neurons, expression of tyrosine hydroxylase.	Augusti-Tocco and Sato, 1969
NT2 (Ntera2)	Human, testicular teratocarcinoma	Serial passage	When cultured in retinoic acid, they form hNT postmitotic neurons. Human cell transplant studies have been conducted.	Andrews, 1984; Andrews et al., 1984; Borlongan et al., 1998; Flax et al., 1998
CNh, CTb	Normal (CNh) and trisomy 16 (CTb)	UCHT1 protocol	Neuronal markers, cholinergic phenotype. Functional neurotransmitter receptors (glutamatergic, cholinergic). Down syndrome related anomalies in CTb cells	Allen et al., 2000, 2002; Arriagada et al., 2000, 2003; Cárdenas et al., 1999, 2002a, 2002b; Cox et al., 1981; Frederiksen et al., 1996; Kim and Hammond, 1995; Paula Lima et al., 2002
RCSN	Adult rat substantia nigra	UCHT1 protocol	Neuronal, dopaminergic phenotype. Functional glutamatergic receptors. Reduces rotational behaviour in 6 OH dopamine rats, after striatal implant.	Arriagada et al., 1998, 2002; Caviedes et al., 2003

this fashion, mouse trisomy 16 cell lines were established from the cerebral cortex (named CTb), hippocampus (HTk), spinal cord (MTh), and dorsal root ganglion (GTI). Simultaneously, cell lines from these same regions were established from normal, age-matched littermates, which serve as controls. These cell lines retain neuronal markers (Neural specific enolase -NSE, Choline acetyl transferase -ChAT, synaptophysin, microtubule associated protein 2 -MAP-2) and lack glial traits (GFAP, galactocerebroside, S-100). Our prior studies have also shown that these cells express functional neurotransmitter receptors, as shown by intracellular  $\text{Ca}^{2+}$  responses to neurotransmitter agonists (Cárdenas et al., 1999), and also cholinergic function (Allen et al., 2000, 2002; Cárdenas et al., 2002a, 2002b), and present alterations of the aforementioned functions in a manner similar to that observed in similar tissues kept in primary culture conditions. Interestingly, CTb and HTk cells overexpress the amyloid precursor protein (Arriagada et al., 2000, 2003; Paula Lima et al., 2002), whose gene is present in both murine chromosome 16 and human autosome 21 conditions. This is considered a major link between Down's syndrome and the characteristics of early onset of Alzheimer's-like pathology in these patients (Epstein, 1986). As such, these cell lines are a potential drug screening tool for anti-amyloidogenic compounds.

Another cell line with potential application to the study and treatment of a neuropathological condition is the RCSN neuronal cell line, derived from the substantia nigra of an adult rat (Arriagada et al., 1998). By immunohistochemistry, the cell line shows positive neuronal markers (NSE, synaptophysin, MAP-2, neurofilament) and while lacking glial traits (GFAP, S100). RCSN cells possess catecholaminergic traits: the presence of TH, melanin, and DAT. This cell line also exhibits intracellular  $\text{Ca}^{2+}$  increments in response to excitatory neurotransmitter agonists (Arriagada et al., 2002; Caviedes et al., 2003). These results suggest the RCSN cell line is a model for the study of cell mechanisms related to Parkinson's like neurodegeneration. Further, using stereotaxic surgery, intra-striatal implanted suspensions of this cell line into rats previously lesioned in the nigrostriatal pathway with 6 OH dopamine, revealed a steady reduction in the typical apomorphine-induced rotational behavior, leveling off at 75%

of the initial rotation values after 16 weeks post implant (Arriagada et al., 2002; Caviedes et al., 2003). The effect of the implanted RCSN cell line in the improvement of the rotational behavior in 6 OH dopamine lesioned rats is encouraging in the quest to establish a human substantia nigra cell line, and thus generate a model that could be applied towards cell transplant therapy in humans. Taken in summary, each of the above cell lines offers significant potential for evaluation of therapeutic candidates.

## DRUG DEVELOPMENT AND CULTURED CELLS

Figure 1 presents a flowchart of the development of cell lines from tissues described herein, and indicates that manipulation targeted to preserve or recover stable, differentiated phenotypes is essential to present a given cell line as a model. Further, Table 1 outlines characteristics of the cell lines described above. In evaluating these cell types, novel therapeutic agents can be tested quickly and rigorously such that the effects of potential drugs can be evaluated and their mechanism identified. Many different aspects of a potential new compound can be evaluated via these cultures. Particularly relevant are cultured cells which represent a disease state model and those which demonstrate pertinent genetic and biochemical abnormalities, as well as alterations in receptors, ion channels, and receptor signaling pathways. It is these aspects of the culture cells which enable significant information about a putative therapeutic agent's usefulness in such pathologies. Hallmarks of disease states which are exhibited in vitro allow for evaluation of abnormality mitigation when compounds of interest are tested in these cultured systems.

In myocardial cells such as those discussed above, it is possible to evaluate the effects of novel agents on parameters yielding beneficial information as to their potential usefulness in cardiac related disease states. Similarly, as with the skeletal cell cultures heretofore described, we can ascertain how new agents will affect aspects of muscle pathophysiology providing insights as to utility for evaluation of drugs for these anomalies. Lastly, assessment of putative new drugs on effects in neuronal cell cultures such as the aforementioned will clearly enable screening and development of therapeutic modalities



that can mitigate the effects of central nervous system disorders. Clearly, these are only a snapshot of potential disorders which can be evaluated in cell culture. The overall potential of agent evaluation in such cultures is significant.

Of equal, if not greater importance, cell cultures afford the opportunity to evaluate not only the positive mitigating effects of novel agents, but they also enable rapid assessment of toxicity profiles in these tissues. Alterations in cell cultures which, when identified, obviously would result in negative effects in the whole animal, provide information that will preclude moving a potential drug into animal testing or, even more important, evaluation in humans can be halted when clear toxicity parameters are identified. There may be no better example of this type of evaluation being critical than evaluating compounds in neuronal tissues/cell culture. For new agents which are being utilized for central nervous system disorders and their effects on neurons, it is paramount that the potential effects of these drugs on the brain be known. Related, agents which have demonstrated utility in other disease states, such as anticancer drugs, but do not normally gain access to brain that have newfound ability to be delivered to the brain must be evaluated for potential neurotoxicity. One very key way to evaluate such is to ascertain the effects of these established therapeutic agents in neuronal cell cultures.

Because it is possible to study tissues from a vast array of animals, including humans, the flexibility to assess how drugs will affect target systems in vitro offers a great advantage early in screening new compounds. Obvious ethical issues related to testing in humans can be circumvented with early evaluation of new agents. Further, if adverse effects are determined in vitro, reduction in the numbers of animals being unnecessarily screened can occur as well. Overall, utility of cell cultures overcomes many obstacles in the drug development and screening processes.

Clearly, many aspects of this review, though focusing on myocardial and skeletal tissues and neuronal cells, pertain to potential utility of drug screening and evaluation for other tissue types, and are particularly relevant to assessing potentially novel therapeutic agents in cancer cells and tumors. The cell culture systems described herein offer significant potential for drug development and screening. Considering Sir William Harvey steered us down a

pathway with the observation that myocardium, “cultured” in the palm of his hand with saliva retained contractile, could be evaluated in vitro, cell culture offers an abundance of promise in drug screening and development.

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