**GROWTH AND DEVELOPMENT SYMPOSIUM:**

**Development, characterization, and use of a porcine epiblast-derived liver stem cell line: ARS-PICM-19**¹²

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**ABSTRACT:** Totipotent embryonic stem cell lines have not been established from ungulates; however, we have developed a somatic stem cell line from the in vitro culture of pig epiblast cells. The cell line, ARS-PICM-19, was isolated via colony cloning and was found to spontaneously differentiate into hepatic parenchymal epithelial cell types, namely hepatocytes and bile duct cells. Hepatocytes form as monolayers and bile duct cells as 3-dimensional bile ductules. Transmission electron microscopy revealed that the ductules were composed of radially arranged, monociliated cells with their cilia projecting into the lumen of the ductule whereas hepatocytes were arranged in monolayers with lateral canalicular structures containing numerous microvilli and connected by tight junctions and desmosomes. Extensive Golgi and rough endoplasmic reticulum networks were also present, indicative of active protein synthesis. Analysis of conditioned medium by 2-dimensional electrophoresis and mass spectrometry indicated a spectrum of serum-protein secretion by the hepatocytes. The PICM-19 cell line maintains a range of inducible cytochrome P450 activities and, most notably, is the only nontransformed cell line that synthesizes urea in response to ammonia challenge. The PICM-19 cell line has been used for several biomedical- and agricultural-related purposes, such as the in vitro replication of hepatitis E virus, a zoonotic virus of pigs, and a spaceflight experiment to evaluate somatic stem cell differentiation and liver cell function in microgravity. The cell line was also evaluated as a platform for toxicity testing and has been used in a commercial artificial liver rescue device bioreactor. A PICM-19 subclone, PICM-19H, which only differentiates into hepatocytes, was isolated and methods are currently under development to grow PICM-19 cells without feeder cells. Feeder-cell-independent growth will facilitate the study of mesenchymal–parenchymal interactions that influence the divergent differentiation of the PICM-19 cells, enhance our ability to genetically modify the cells, and provide a better model system to investigate porcine hepatic metabolism.

**Key words:** bile duct, cell line, differentiation, hepatocyte, liver, stem cell

**INTRODUCTION**

In vitro models of mammalian tissues have been very important for studying the cellular and molecular processes that enable multicellular life to exist and function. Equally important has been the contribution of in vitro models to biomedical knowledge and applications. Most often, in vitro models consist of cell cultures established as a more or less 2-dimensional (2D) monolayer of cells maintained or continuously grown in flasks or plates. The cell cultures can be so-called “primary cell cultures,” created for 1-time use from freshly isolated cells, or can be “cell lines,” which are distinguished from primary cultures by the ability of the cells to replicate for various lengths of time. Cell lines, as in vitro models, are also defined by having a homogenous cell population of a particular cell type, the cells of which remain relatively stable in phenotype...
as they grow over time. Finite cell lines may expand in cell number, that is, be continuously cultured from flask to flask, for months or years but will eventually cease dividing and become senescent. In contrast, immortal cell lines, as the name implies, can be continuously cultured indefinitely, and this special property makes such cell lines useful for creating stable mutations in the cells by either genetic engineering technology or application of selective pressures of various kinds. Immortal cell lines, therefore, have been and will continue to be extremely important tools for studying the biology and pathology of the various tissues and organs of the body.

The cell source for creating in vitro models has traditionally been the isolation of cells from the particular tissue or organ of interest, or, for immortal cell lines, the cell source has frequently been tumors of a particular tissue or organ. However, this changed after the establishment of embryonic stem cell (ESC) lines from the mouse (Evans and Kaufman, 1981; Martin, 1981) and from monkeys and humans (Thomson et al., 1995, 1998) because ESC can theoretically differentiate into any cell type in the body, that is, they are totipotent. Similar to ESC, recent breakthroughs in transgenic manipulation of somatic cells to produce induced pluripotent stem cells or direct conversion to other somatic cell types has created another source from which to make in vitro cell models and cell lines (Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Han et al., 2012). In this same context, it is possible to derive cell lines from the primary culture and spontaneous differentiation of the epiblast tissue of an early embryo, the epiblast being the totipotent tissue of the early embryo that can give rise to ESC lines (Brook and Gardner, 1997). Herein we describe 1 such epiblast-derived immortal cell line, the PICM-19 cell line, isolated from the culture of porcine epiblast cells and shown to be a bipotent liver stem cell line.

**ISOLATION AND CHARACTERISTICS OF THE PICM-19 PIG LIVER CELL LINE**

**Origin from Pig Epiblast Culture**

The PICM-19 cell line was derived from an 8-d-old porcine (*Sus scrofa domestica*) embryo flushed from the uterus of a female pig in October 1991. The inner cell mass (ICM) of this early blastocyst-stage embryo was isolated by immunodissection, using an anti-pig-spleenocyte polyclonal rabbit antiserum, and the ICM was cultured on Sandoz inbred strain, thioguanine- and ouabain-resistance (STO) feeder cells (irradiated mouse fibroblasts) and before differentiation at 200x magnification. The PICM-19 cell line was derived from an 8-d-old primary colony as illustrated in (B) at 200x magnification. Panel C: Nascent colony (arrows) of PICM-19-like liver stem cells after selection and cell cloning from a differentiated primary pig epiblast colony as illustrated in (B) at 200x magnification. Panel D: Differentiated PICM-19 cell culture; note formation of 3-dimensional bile ductules (arrows) and of monolayer of hepatocytes with biliary canaliculi visible between some of the cells (arrowheads); at 200x magnification.

**Growth Characteristics**

The medium and reagents used to currently grow and maintain the cells are similar to those used at the inception of the cell line, as described in detail (Talbot and Paape, 1996). The STO mouse fibroblasts (CRL1503; American Type Culture Collection, Manassas, VA) are used as feeder cells in the culture of PICM-19 and are rendered incapable of replication by exposure to 10 mg/mL mitomycin C for 4 h or to 80 Gy γ radiation (Talbot and Paape, 1996; Talbot et al., 2000).
Like hepatic cell lines derived from tumors or cell lines immortalized by incorporation of oncogenes (i.e., transformed cells), PICM-19 cells have the ability to replicate indefinitely; however, unlike tumor-derived or transformed cell lines, PICM-19 cells largely stop replicating 10 to 14 d after being passaged, and the cells differentiate into hepatocyte monolayers or bile ductules, as would normally occur in regenerating liver in vivo (Fig. 1D). This is exemplified by the cells never reaching a confluent state in the culture flask, that is, covering all of the available growth surface (Talbot et al., 1994b). Because of this intrinsic differentiation characteristic, and like other stem-cell lines (e.g., ESC), every 10 to 14 d cultures of the PICM-19 cells must be dissociated to a suspension of single cells by treatment with trypsin and EDTA and then re-plated onto fresh STO feeder cells; this maintains their growth potential and enables their continuous culture over time. Without this periodic cell-to-cell dissociation, the PICM-19 cells will terminally differentiate.

The PICM-19 cells grow relatively slowly with a doubling time of 48 to 72 h, and their growth can be affected by the addition of growth factors (Fig. 2). When added to the culture medium, some cytokines and growth factors stimulate growth of the PICM-19 cells, specifically, leukemia inhibitory factor, granulocyte-macrophage colony-stimulating factor, IGF-1, and hepatocyte growth factor, whereas others are inhibitory or promote cell death, for example, epidermal growth factor and tumor necrosis factor-α, respectively. Transforming growth factor-β, type I, is also inhibitory to PICM-19 cells (Talbot et al., 1994b).

Until recently, PICM-19 cells were dependent on contact with the STO feeder cells for their growth and for their maintenance of hepatocyte and bile duct epithelium cell functions. Maintaining hepatocyte-specific cell function in vitro is a universal problem in all primary cultures of normal hepatocytes, that is, cultures of hepatocytes freshly isolated from liver tissue. Primary hepatocyte cultures can lose their hepatocyte-specific functions in as few as 48 to 72 h of in vitro culture unless they are cultured on a biomatrix (Dunn et al., 1989) or co-cultured with feeder cells (Caperna et al., 2011), and PICM-19 cells share this normal hepatocyte characteristic. However, it is now possible to continuously culture PICM-19 cells on a substrate of polymerized type I collagen and with an overlay of diluted (1:50) extracellular matrix (e.g., Matrigel; BD Biosciences, Bedford, MA), as long as the medium is separately conditioned by exposure to STO feeder cells (Talbot et al., 2010a; Talbot and Caperna, 2013). The feeder-independent culture of PICM-19 cells is discussed in more detail below.

**Bipotent Stem Cell Nature**

The cell line is unique in its ability to differentiate into either of the 2 cell types that make up the parenchyma of the developing liver: fetal hepatocytes or cholangiocytes, that is, bile duct epithelium (Talbot et al., 1994a, 1994b; Talbot and Caperna, 1998). When grown on STO feeder cells, PICM-19 cells differentiate into either monolayers of fetal hepatocytes or into functional bile ductules with self-organization into multicellular, 3-dimensional (3D) tubular structures (Fig. 1D; Talbot et al., 1994b, 1996a, 2002). The PICM-19 hepatocytes have the characteristic morphology of fetal and neonatal pig hepatocytes, that is, cuboidal cells with centrally located nuclei and joined laterally by tight junctions and desmosomes to form biliary canalicular structures between the cells (Fig. 3A and 3B; Talbot et al., 1994a, 1996a). The PICM-19 biliary canaliculi are filled with microvilli, indicating the lateral polarized nature of the cells (Fig. 3B). Their nuclei are typically indented, and their cytoplasm contains numerous mitochondria, abundant rough and smooth endoplasmic reticulum and Golgi apparatus, and nearly empty-appearing spaces where the remnants of glycogen rosettes can be seen (Fig. 3A and B). The PICM-19 cells that differentiate into bile ductules assume a radial arrangement of 5 or 6 cells with...
tight junctions between neighboring cells so as to form a central lumen (Fig. 3C). The luminal surfaces of the ducts are ciliated, probably with monocilia, and have relatively few microvilli present (not shown; Talbot et al., 1994b, 1996a, 2002). These in vitro-produced bile ductules closely resembled similar bile ductules that were produced in primary cultures of liver cells isolated from both fetal and adult pig liver tissue and co-cultured on STO feeder cells (Talbot et al., 1994a; Talbot and Caperna, 1998; Caperna et al., 2011). The ultrastructural features of the PICM-19 bile duct epithelial cells are similar to those of PICM-19 hepatocytes in monolayer except that numerous mucus containing vesicles are often observed within the cytoplasm, particularly just below the apical (luminal) cell membrane (not shown; Talbot et al., 2002; see also PICM-19B in Talbot et al., 2010c).

It has been observed that the differentiation of the PICM-19 cells, both in phenotype and extent, is affected by variation in some basic culture parameters. These are pH of the culture medium, concentration of irradiated STO cells making up the feeder layer, and the height of the medium over the cells, which presumably relates directly to the relative concentration of dissolved oxygen at the level of the cell monolayer (Pettersen et al., 2005; Oze et al., 2012). The pH of the medium dictates the type of liver cell achieved in the culture, hepatocyte or cholangiocyte, and the density of the feeder cells and the height of the medium in the flask or dish dictates the tendency for the cells to drop out of the cell cycle and terminally differentiate. Specifically, a relatively low culture pH of approximately 7.0 biases the PICM-19 cells toward forming a hepatocyte monolayer, and a relatively high pH of approximately 7.8 causes the formation of cholangiocytes and 3D bile ductules. By this means, it is possible to create PICM-19 cultures that are largely devoid of 3D ductule structures or, conversely, to create PICM-19 cultures that are composed of a “web” of interconnected ductules with very few hepatocytes present (Talbot et al., 2002). A minimum density of feeder cells, approximately $25 \times 10^3$ cells/cm$^2$, is required for the PICM-19 cells to maintain differentiated form and function as either hepatocytes or cholangiocytes and concentrations of feeder cells $>50 \times 10^3$ cells/cm$^2$ decreases cell proliferation in favor of terminal differentiation (N.C. Talbot and T.J. Caperna, unpublished data). The phenomenon of medium height affecting the relative proportion of PICM-19 replication vs. differentiation is dramatic but to date has not been investigated beyond simple microscopic observation. That the effect is probably the result of the relative concentrations of gases in the medium is supported by placing a flask of recently passaged PICM-19 cells on a slight incline; over a subsequent 10 to 14 d in culture, the “deep end” of the flask...
is seen to have a sparse grouping of terminally differentiated PICM-19 cells whereas the cells at the “shallow end” will have grown to a nearly confluent monolayer of differentiated or differentiating PICM-19 cells. This observation would, therefore, appear to preclude autocrine- or paracrine-secreted molecules as causing the effect (Przybyla and Voldman, 2012).

**Hepatocyte and Bile Duct Functions In Vitro**

The PICM-19 cells display functional attributes typical of fetal and adult hepatocytes including serum-protein production, inducible P450 content and activity, ammonia clearance, and urea production (Talbot et al., 1994b, 1996a, 2010c; Caperna et al., 2010, 2011). The relative concentrations of serum proteins secreted into the medium by the PICM-19 cells is similar to that found in the serum of the 50-d pig fetus in that α-fetoprotein (AFP) is produced along with a small amount of albumin. Similarly, a relative abundance of acidic glycoproteins and transferrin are secreted by PICM-19 cells and the early fetal pig liver in comparison with their decreased concentrations found in the serum of the 100-d pig fetus and the newborn pig (Talbot et al., 1994b, 2010c; Caperna et al., 2010). The PICM-19 ductal structures strongly express γ-glutamyltranspeptidase, an enzymatic marker of bile duct epithelium, at their luminal surfaces (Tanaka, 1974; Ishii et al., 1989) and respond to physiological levels of secretin (picomole amounts) by basolateral to apical transport of culture fluid with in vivo-like kinetics (Fig. 4, USDA weblink; Talbot et al., 2002).

**Subclones of the PICM-19 Cell Line**

Several subpopulations of the PICM-19 cell line have been established in recent years with particular cell phenotypes or for particular culture purposes. The PICM-19H cell line was isolated after 3 to 4 wk of hypothermic culture (~33°C) in which greater than 99% of the cells died out. It was observed that the cells that survived no longer exhibited spontaneous self-organization into bile ductules when grown on STO feeder cells, even after being held in static culture for several months with refeeding every other day (Fig. 5). The PICM-19H cell line, therefore, is a homogeneous culture of fetal porcine hepatocytes. The PICM-19H cells exhibit the hepatocyte functions of serum-protein production, several P450 activities, ammonia clearance, and urea production and have reduced glutamyltranspeptidase activity in comparison with the parental PICM-19 cell line (Talbot et al., 2010c). Instead of terminally differentiating before a confluent monolayer is formed, as with the PICM-19 parental cells, the PICM-19H cells were found to grow to confluence and, therefore, were primarily controlled by the phenomenon of contact inhibition. This property allows the cells to grow to greater cell numbers per culture flask than the parental cells. The PICM-19H cells are also easier to dissociate from one another with trypsin and EDTA treatment than the parental cells and can be passaged at greater passage ratios (typically 1:5 or 1:10) than the parental cells. Taken together, these cell and culture characteristics make the PICM-19H cell line better for some potential applications than the parental PICM-19 cell line, as discussed subsequently.

A spontaneously occurring phenotypic variant of PICM-19 cells was colony cloned to yield a new cell line referred to as PICM-19B (Talbot et al., 2010c). The PICM-19B cells appear to arise from PICM-19 parental cells undergoing bile duct differentiation, but instead of terminally differentiating into multicellular ductule struc-
PI CM-19 pig liver cell line

The PICM-19B variant cells keep growing until a confluent, tightly knit, monolayer sheet of epithelial cells is formed in the culture vessel. The PICM-19B cells of the cell sheet are basolaterally polarized with tight junctions between the cells and abundant microvilli displayed on the uppermost (i.e., facing the medium of the flask) apical cell membrane; that is, biliary canaliculi are not present between the cells (Talbot et al., 2010c). The cells also possess monocilia projecting out of the apical membrane and into the culture medium, and cytoplasmic bodies resembling mucus secretory granules are often observed underneath the apical membrane (not shown; Talbot et al., 2010c). The PICM-19B cells display relatively high levels of γ-glutamyltranspeptidase activity, low levels of P450 activity, and relatively low ammonia clearance and urea production, and they secrete almost no serum proteins except for trace amounts of AFP, retinol-binding protein, and transferrin (Talbot et al., 2010c). As with the PICM-19H cells discussed previously, the PICM-19B cell line is easier to dissociate to a near-single-cell suspension with trypsin and EDTA, it produces 2 to 3 times as many cells per flask as the parental PICM-19 cells, and it can be passaged at passage ratios of 1 to 5 or 1 to 10, unlike the parental cells that are routinely passaged at 1 to 2 or 1 to 3 passage ratios. It is anticipated that the PICM-19B cells will be useful for vectorial transport measurements, for example, use with cell culture membrane inserts for basolateral cell transport studies of bile duct epithelium.

The goal of growing the PICM-19 cells without feeder cells and in a semidefined medium is being pursued. The first part of this goal, the continuous culture of the parental PICM-19 cells without contact with a feeder layer of STO cells, was recently achieved (Talbot et al., 2010a). The culture system uses a combination of a polymerized collagen type I thin-layer substrate, a diluted Matrigel overlay, and the use of STO feeder-cell-conditioned medium. This allows the PICM-19 cells to be manipulated and assayed without the confounding presence of the mouse STO feeder cells. The first feeder-independent cultures of PICM-19 cells, designated PICM-19FI, unfortunately reached a senescent endpoint after about 18 passages and over this passage history grew to relatively low concentrations per unit culture area (Talbot et al., 2010a). Subsequently, however, a spontaneously arising population of feeder-independent PICM-19 cells arose from this senescing cell population. These cells, designated PICM-19FF, have been extensively passaged, that is, passaged every 2 wk at a 1 to 3 passage ratio for over 2 yr and, therefore, appear to be functionally immortal. The PICM-19FF cells grow to confluency in 10 to 12 d after passage and display normal hepatocyte morphology with cyclic AMP-responsive bile duct epithelium.

**Figure 5.** PICM-19H 4 mo old culture (Panel A) compared with primary adult pig hepatocytes co-cultured on Sandoz inbred strain, thioguanine- and ouabain-resistance (STO) feeder cells (Panel B). Magnification = 200x. Note biliary canaliculi between cells (arrowheads).

**POTENTIAL USES OF THE PICM-19 CELL LINE**

**Liver Biology Model**

The PICM-19 cell line and its derivative cell lines are relevant to the study of stem cell biology in general, liver development and differentiation, and, perhaps most importantly, as in vitro models for hepatocyte and cholangiocyte cell biology. Although many other hepatocyte primary culture systems and cell lines from various animals exist (Strick-Marchand and Weiss, 2003; Parent et al., 2004; Kia et al., 2012), the PICM-19 cell line is unique in being a nontransformed, that is, noncancerous, yet immortal cell line that spontaneously differentiates into both normally functioning fetal hepatocytes and cholangiocytes. This occurs in a 2D-culture context, that is, without the use of specialized, cumbersome, and variable 3D substrate systems such as biogels.
composed of collagen or extracted extracellular matrix (e.g., Matrigel). Thus, the PICM-19 cells can be grown in culture indefinitely and maintain their liver cell specific differentiation potential and functions.

The analysis of the ultrastructure, fluid transport, metabolic functions, and gene expression of the PICM-19 cells demonstrated characteristics of both parenchymal hepatocytes and bile duct epithelium cells (i.e., cholangiocytes). The findings are consistent with the PICM-19 cell line as a liver stem cell line because both hepatocytes and cholangiocytes are thought to come from a common precursor cell derived from the definitive endoderm of the primitive gut (Wilson et al., 1963). Embryological data based on morphological characteristics (Enzan et al., 1974) and immunohistochemical analysis of cytokeratins (Van Eyken et al., 1988) indicate that intrahepatic biliary epithelial cells arise from hepatoblasts or parenchymal cells. It has been concluded that embryonic parenchymal liver cells possess characteristics of both hepatocytes and cholangiocytes (Shah and Gerber, 1989), and phenotypic plasticity between hepatocyte and cholangiocyte has been shown in vitro (Block et al., 1996; Nishikawa et al., 2005). Evidence also exists for liver stem cells in the adult animal (Brill et al., 1993; Cardinale et al., 2011). The proposed liver stem cells are postulated to be able to give rise to both hepatocytes and cholangiocytes and to be of fetal phenotype, for example, express AFP, as do PICM-19 cells, and recent research has better defined the nature of the parenchymal stem cell niche in the developing liver and the adult liver (Cardinale et al., 2011; Turner et al., 2011). Thus, the collected observations and data on PICM-19 cells and parallel studies conducted on fetal and adult pig liver cells in co-culture with STO feeder cells (Talbot et al., 1994a; Talbot and Caperna, 1998; Caperna et al., 2011) support the proposal that the PICM-19 cell line is an in vitro equivalent of the progenitor cells of the embryonic liver or the hypothesized adult liver stem cell.

The PICM-19 cell line offers many opportunities for studying the biology and pathology of hepatocytes and cholangiocytes and, by extension, of the animal liver as a whole. As mentioned previously regarding feeder-cell-free propagation of the cells, the PICM-19 cell line could be a useful model for studying the mechanisms that control ductule differentiation and formation in the liver. Also, because the PICM-19B cells appear to contain numerous mucin secretory granules, the cell line might be used as an in vitro model of the bile duct pathology associated with cystic fibrosis (Herrmann et al., 2010) and certain cancers of the liver (Cardinale et al., 2012). To date, the PICM-19 cell line has been used, to greater or lesser extents, for several biomedical related purposes. These include tests of the in vitro propagation of the protozoan agents of malaria and toxoplasmosis, testing replication of hepatitis viruses (particularly hepatitis E virus, a zoonotic virus of pigs; Rogée et al., 2012), evaluating gene therapy vectors targeting the liver and using the AFP promoter, cell transformation studies with oncogenes for liver cancer modeling, and a spaceflight experiment to evaluate the effects of microgravity on somatic stem cell differentiation and liver cell function (Talbot et al., 2010c). Most recently, investigations of fatty acid synthesis and triglyceride storage in PICM-19 cells have begun to see if the cells could be an in vitro model for fatty liver disease in people (Reddy and Rao, 2006; Stoll et al., 2010). The PICM-19 cells accumulated large amounts of lipid under certain culture conditions, as do adult pig hepatocytes grown on STO.
feeder cells (Talbot et al., 1994a,b; N.C. Talbot and T. J. Caperna, unpublished data). The most extensive evaluations for using the PICM-19 cell line have been in its assessment and development for in vitro toxicological and pharmacological testing and for its use in an extracorporeal artificial liver device for treatment of acute liver failure in humans.

**Toxicology and Pharmacology Models**

Improved in vitro models of in vivo liver biotransformation and toxicity are needed to enable faster biological evaluation of new chemical entities (NCE) and to reduce controversial and costly animal testing (Bertz and Granneman, 1997; Guillouzo, 1998; Yan and Caldwell, 2001). Presently, most in vitro testing of NCE for adverse reactions with liver cells is performed with primary hepatocyte cultures, transformed hepatocyte cell lines, or microsomal preparations derived from liver tissue or cells (Bertz and Granneman, 1997; Yan and Caldwell, 2001; Vermeir et al., 2005). Microsomal preparations, although useful for some assessments, cannot be used to model cellular enzyme inductions, transport processes, or toxicity (Shimada et al., 1994; Gómez-Lechón et al., 2004). Primary hepatocyte cultures provide models for liver cellular function and can be prepared from a variety of species, including from specific disease states (Guillouzo, 1998; Ulrichova et al., 2001; Gómez-Lechón et al., 2004). However, hepatocyte preparations are limited in their growth and survival in vitro, necessitating the continual acquisition of hepatocytes from livers (Guillouzo, 1998; Hoekstra and Chamuleau, 2002; Rodriguez-Antona et al., 2002). Good quality human liver tissue is frequently in short supply and must be handled as if infectious (Guillouzo, 1998; Hoekstra and Chamuleau, 2002). Animal liver tissue can be obtained in quantity and is usually not an infectious disease hazard. However, whether derived from human or animal tissue, culture quality can vary widely due to genetic variation, health, nutritional status, stress levels, and the skill of the cell culturist in preparing the hepatocyte cell suspension (Guillouzo, 1998; Di Niculo et al., 2005). Liver cell models based on hepatocyte cell lines that grow continuously, that is, are functionally immortal, can address these problems. Unfortunately, these cell lines, tumor derived or transformation derived, are functionally abnormal due to their characteristics of unabated growth and lack of normal differentiation, and they therefore make poor models. In particular, phase I and II enzymatic reactions and cellular transport properties, used as a basis for estimating in vivo toxicokinetics and pharmacokinetics, are diminished (Guillouzo, 1998; Yan and Caldwell, 2001; Hoekstra and Chamuleau, 2002; Wilkening et al., 2003). Given these drawbacks, a cell line that exhibits unlimited growth and yet differentiates normally; for example, a liver stem cell line, may be the best in vitro model with which to obtain repeatable and standardized pharmacological and toxicological assessments of NCE.

The PICM-19H derivative cell line is a good candidate for the in vitro toxicological and/or pharmacological assessments of NCE before in vivo liver toxicology and pharmacology studies are performed. Recently, the PICM-19H cell line was characterized to show the presence and induction of the major cytochrome P (CYP) 450 family members and the extent of phase II conjugations. The hepatic CYP450 system is associated with detoxification of xenobiotics as well as the metabolism of endogenous molecules including cholesterol and associated steroids. Treatment of PICM-19H cells with specific inducers and measurement of substrate metabolism was indicative of CYP450 family members CYP1A1, CYP1A2, CYP2, and CYP3A (Willard et al., 2010). Also, although testosterone metabolism in noninduced PICM-19H cultures was negligible, rifampin pretreatment of the cells resulted in a greater than 10-fold induction of hydroxylated metabolite formation. The PICM-19H cells produced 6b-hydroxy testosterone as the predominant hydroxylated species, the same as found for pig liver microsomes and fresh pig hepatocytes (Donato et al., 1999; Willard et al., 2010). Very recently, feeder-free PICM-19 cell cultures, the PICM-19FF cell line, were assayed for demethylase CYP450 activities by pretreating the cells with a cocktail of rifampicin, 3-methylcholanthrene, phenobarbital, and dexamethasone. The preliminary results showed demethylase activities similar to in vivo levels (Nebbia et al., 2001) with specific induction of erythromycin N-demethylase activity and potential induction of mononesin O-demethylase activity (Fig. 7). Although similar CYP450 demethylase activity levels for aminopyrine, dextromethorphan, and naproxen substrates were observed, these activities were apparently not induced by exposure of the cells to the chemical cocktail (Fig. 7). Phase II reactions are mediated by cellular enzymes that are responsible for detoxification of xenobiotics via conjugation with water-soluble chemical moieties. This key function enhances the elimination of xenobiotic compounds from the body. With both coumarin- and resorufin-based substrates, PICM-19H cells demonstrated phase II conjugation reactions that were comparable to primary cultures of adult pig hepatocytes (Willard et al., 2010). It has been confirmed that the phase II activities of sulfation and glucuronidation are expressed in pig liver as they are similarly expressed in human liver (Diaz and Squires, 2003). Also, known hepatotoxins, acetaminophen and aflatoxin B1, were shown to be metabolized in a dose-dependent manner by PICM-19H cells (Willard et al., 2010). These data com-
bined with the other demonstrated hepatic differentiated functions and characteristics discussed above indicate that NCE might be usefully assayed with PICM-19 cells. Because PICM-19 cells are relatively robust and easily manipulated with standard cell culture reagents, the cells could be applied to automated, multiwell, testing platforms for the rapid assessment of thousands of NCE.

**Bioartificial Liver Device**

Cell lines that possess in vivo-like hepatocyte functions are needed for the biological component of bioartificial liver devices (BLD) that are currently in development (Strain and Neuberger, 2002; Chamuleau et al., 2005; Carpentier et al., 2009). Tumor-derived cell lines, of human or animal origin, are without exception compromised in their liver functions, presumably because of their lack of normal differentiation and uncontrolled growth characteristics (Nyberg et al., 1994; Wang et al., 1998; Rodríguez-Antona et al., 2002; Filippi et al., 2004). Although new cell lines transfected with immortalizing transgenes are being developed and tested, there is no assurance that these cell lines will not suffer from similar problems for similar reasons (Hoekstra and Chamuleau, 2002; Filippi et al., 2004). Qualities that make the PICM-19 cell lines more favorable for application to a BLD are that they retain critical hepatocyte functions, they are nontumorigenic and display normal differentiation in vitro, they may be maintained in the bioreactor of the BLD for relatively long periods of time, their phenotypic stability and pathogen-free status can be defined and routinely assessed, they can be genetically engineered for enhancement of function and protection from preformed antibodies, and finally, as described previously, natural or induced mutant subcell lines may be developed and assessed for potential improved function in a BLD (Talbot et al., 1994b, 1996a, 2010c; Willard et al., 2010).

Under a Cooperative Research and Development Agreement and U.S. patents number 5,532,156, “Hepatocyte Cell line Derived from the Epiblast of Pig Blastocysts,” (Talbot et al., 1996b) and number 5,866,420, “Artificial Liver Device,” (Talbot et al., 1999) the PICM-19 cells were licensed to HepaLife Technologies, Inc. (Boston, MA) and subsequently to HepaLife Biosystems, Inc. (a division of Alliqua, Inc., New York, NY) for research and development of a BLD for use on humans suffering acute liver failure. A working flow-through 3D-bioreactor system was developed containing PICM-19H cells. The system was tested several times for in vitro function endpoints such as cell survival, albumin production, ammonia clearance, and urea production over 2- to 3-wk operational periods (Willard et al., 2008). Future research initiatives will be to optimize the growth and long-term maintenance of PICM-19 cells in a 3D culture environment with continuous, flow-through perfusion of medium.

**Summary and Conclusions**

The USDA ARS has developed several in vitro model systems to investigate various aspects of porcine hepatic gene expression and metabolic regulation. These systems encompass both established cell lines and primary liver cell cultures. One porcine hepatic stem cell line, derived from porcine epiblast (ESC tissue) is the ARS-PICM-19 cell line, which has been partially characterized and is a nontransformed immortal cell line that possesses many characteristics similar to that of intact liver parenchymal cells. The ARS is interested in further characterization and improvements in the culture technology that would ultimately result in the cell line not
These advancements would facilitate the understanding vectors and for research on zoonotic diseases shared by pigs and humans. It is also hoped that the PICM-19 cell line will have application for use in the production of a rescue device for human patients in liver failure. The PICM-19 cell line illustrates the potential for deriving unique somatic cell lines or somatic stem cell lines from the primary culture of the totipotent epiblast tissue of pigs and, by extension, other farm animals.

LITERATURE CITED


