Establishment and Characterization of USM-BC-1 Cell Line derived from Human Urinary Bladder Cancer

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Abstract

Transitional cell carcinoma of the urinary bladder is one of the common diseases that causes morbidity and mortality worldwide. In Malaysia, bladder cancer is in the 10 most frequent cancer occurred among males. Since most of the current urinary bladder cancer cell lines were established from populations in western countries while significant genotypic variations have been reported among various populations, this project aimed at establishing the first continuous cell line for providing an ideal cancer cell model for Malaysian population and nearby countries acquires significance. The urinary bladder cancer cells were isolated from bladder cancer tissues obtained from a Malay patient. The obtained cells were cultured continuously over 30 passages. This cell line was characterized by observing its morphology, growth characteristics, presence of cytokeratin 7 and desmin markers, expression of Ki-67, VEGF as well as p21 proteins. The authenticity of the obtained cells was investigated using Short Tandem Repeat (STR) analysis and contamination by Mycoplasma was ascertained. Results revealed that this newly established cell line (USM-BC-1) has been well characterized as an ideal cancer cell model which may prove useful in cancer research towards developing future diagnostic and therapeutic targets, taking into consideration the genotypic variations among Asian population.

KEYWORDS: Urinary Bladder Cancer, Cell line, USM-BC-1, Establishment, Characterization

INTRODUCTION

Being one of the significant causes of morbidity and mortality worldwide [1], transitional cell carcinoma of the urinary bladder has been ranked in the 10 most frequent cancer observed among Malaysian males [2]. Pertinently, tumor cell lines are valuable resource materials in cancer research, drug discovery and other works in biomedicine [3-5]. Since the first establishment of human urinary bladder cancer cell lines in 1970, more than 50 human urinary bladder cell lines have been reported [6]. Majority of the bladder cancer cell lines were established a few decades ago among Caucasian populations [7]. A review of the American type culture collection (ATCC) database shows that the human urinary

bladder cancer cell lines deposited from Asian populations remain sparse. Since those cell lines were old and perhaps established in the different environmental condition from the current highly industrialized world as well as differences in neoplastic cells behavior attributable to ethnicity have been reported [7], it is found pertinent to establish new human urinary bladder carcinoma cell lines in Malaysia. The establishment of this new cell line would prove useful in investigating the genetic and epigenetic aspects that trigger the progression of urinary bladder cancer in Malaysia as well as in other Asian populations in nearby countries.

This project utilized a tissue sample obtained from a Malay patient who underwent a radical cystoprostatectomy for urinary bladder cancer at the Hospital Raja Perempuan Zainab Kota Bharu, Kelantan to establish the first ever human urinary bladder carcinoma cell line (USM-BC-1) in Malaysia.

MATERIALS AND METHODS

Cell culture

Fresh tissue specimen of third stage urinary bladder cancer (about $1cm^3$) was sampled from a male patient aged 70 years old. The specimen was brought to the laboratory in a sterile falcon tube (50 cm^3) filled with RPMI-1640 medium (15 cm^3, Thermo scientific, USA) that contained fungizone and penicillin-streptomycin (1%), (Invitrogen, California). The tube was kept at 4°C prior to processing at about 18 hours later. After removing the non-tumor as well as necrotic tumor tissues using sterile opthalmological tweezers, the tumor tissue was minced into small pieces in a sterile petri dish followed by isolation and purification using cancer cell isolation and purification kit (Panomics, USA) following the prescribed protocol. The obtained cell line was cultured in a 25 cm^2 flask that contained RPMI-1640 medium and fetal bovine serum (FBS, 10%, Thermo Scientific, USA) followed by incubation at 37°C with the presence of CO_2 gas (5%). In this research, the RPMI-1640 medium added with penicillin-Streptomycin (1%) and FBS (10%) was referred as the complete medium. For sustaining the growth of the cells, the complete medium in the flask was changed at every three days interval until confluenced growth was achieved. Upon confluenced growth, the cells were washed for three times using sterile phosphate buffer solution (PBS, Bio-rad, USA) prior to the addition of one mL of trypsin-EDTA (0.25%, Invitrogen, California) solution for enabling the subsequent subculturing process at the ratio of 1:2 to 1:4. For further use, the viability of the cells grown was maintained by preserving them in cryovials that contained FBS (90%) and dimethyl sulfoxide (DMSO, 10%) and kept at -180°C. The newly established USM-BC-1 cell line was characterized by investigating the growth characteristics (population doubling time, attachment and plating efficiencies of the cell line), presence of cytokeratin 7 and desmin markers, expression of Ki-67, vascular endothelial growth factor (VEGF), p21 protein, Short Tandem Repeat (STR) analysis and contamination by Mycoplasma.

Mycoplasma contamination test

Contamination by Mycoplasma would alter the cellular metabolism, morphology of cells, genetics and protein expression, signal transduction and proliferation characteristics of cells [8] hence, for practical application it is crucial to ensure that our newly established

cell line was free of such contamination. For this, the commercially available Mycoplasma species PCR kit (Gene Proof, Czech Republic) was used following the standard manufacturer protocol. The kit enabled qualitative detection of various *Mycoplasma* species namely *Acholeplasma axanthum, Mycoplasma alvi, Mycoplasma arginini, Mycoplasma buccale, Mycoplasma cavipharyngis, Mycoplasma cloacale, Mycoplasma fastidiosum, Mycoplasma fermentans, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma salivarium, Mycoplasma pulmonis, Mycoplasma orale, Mycoplasma penetrans, Mycoplasma pirum, Mycoplasma pneumoniae, Ureaplasma diversum, Ureaplasma parvum, Ureaplasma urealyticum* and *Acholeplasma sp*. The PCR results were analyzed using gel electrophoresis (2% agarose gel) and the presence of a specific band of 270 bp would indicate positive detection of Mycoplasma species.

Growth Characteristic

Population doubling time (PDT)

An initial concentration of about 1.5×10^4 cells was added in each of the 24-well plate that contained complete medium followed by the incubation at 37°C with the presence of CO_2 gas (5%) for about 24 hours. Firstly, cells in three of the 24-wells were trypsinized and the number of cells in 20 µL of the cell suspension stained with the equal volume of trypan blue solution in each well was counted using a hemocytometer. Under light microscope, the viable and dead cells appeared transparence and blue, respectively. The same procedure was repeated with the remaining wells every day over the span of the next 7 days. The overall growth of viable cells throughout the observation period of eight days was plotted and the PDT was determined. The PDT was determined from the slope at the linear stage of the mid exponential phase, from the following formula; PDT= $\frac{In\,(N/No)}{t}$ where PDT is the population doubling time, ln is the natural log of the number, N is the final cell number, N_o is the initial cell number and t is the time interval between N_o and N.

Attachment and plating efficiencies

For evaluating the attachment efficiency of the cells cultured, seeding cells (3.5×10^4) were added to a T-75 flask containing 10 mL of complete medium. After 12 hours of incubation, the medium was aspirated, while the attached cells in the flask were rinsed with PBS, trypsinized and counted. This procedure was conducted in triplicates and the mean values were calculated. On the other hand, the plating efficiency of the cells cultured was determined by seeding about 100 cells into a T-75 culture flask that contained 10 mL of complete medium and incubated for 10 days for allowing the cells to grow into colonies. Upon completion, the medium was aspirated, while the attached cells were rinsed using PBS, fixed using 95% ethanol and stained with Harris Hematoxylin dye. The number of colonies that made up of ≥50 cells remained in the flask were counted.

Immunocytochemistry

To confirm the epithelial phenotype and urothelial origin, the USM-BC-1 was characterized using immunocytochemistry technique by evaluating the presence of

cytokeratin 7, while the absence of desmin antibody would verify the absence of fibroblast. Prior to employing the immunocytochemistry technique, the cells were cultured in a four-well camber slide until they became confluenced followed by the fixation process using cold methanol (about 4°C) and kept at constant temperature of 4°C for 20 minutes. The immunocytochemistry technique was performed using a commercial kit i.e. LSAB+system-HRP (Dako, Denmark), as per protocol prescribed by the manufacturer.

Western blot

Western blot technique was performed to study the expression of Ki-67, VEGF and p21 proteins of the newly established USM-BC-1 cell line. Firstly, crude proteins were extracted using radioimmunoprecipitation assay buffer (RIPA, Amresco, USA), kept at constant temperature of 4°C overnight followed by performing the SDS-PAGE technique for separating the different sizes of proteins. Upon completion, the expressions of the desired proteins namely Ki-67, VEGF and p21 proteins were studied. The SDS-PAGE and western blot techniques were performed the following methods prescribed by Walker (1996). Three primary antibodies viz. mouse monoclonal Ki-67, mouse monoclonal VEGF and mouse monoclonal p21 (Santa Cruz, USA) were used at dilution factors of 1:1000, 1:1000 and 1:500 respectively. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Santa Cruz, USA) was used as the secondary antibody at a 1:2000 dilution. Antibody binding was detected using Immobilon Western Chemiluminescent HRP Substrate (MILLIPORE, USA).

STR analysis

Since this research involved the establishment of a new cell line derived from a donor's bladder cancer tissues through many stages of subculturing that may acquire cross-contaminations, it was found pertinent to ensure the authenticity of the obtained cell line via STR analysis. Prior to the STR analysis, the DNA content in both the USM-BC-1 and donor's cancer tissues was extracted using the DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer's protocol. Upon completion, the extracted DNA was transferred into a sterile eppendorf tube and stored at -20°C prior to further analysis. For studying the polymorphism of the STR, several well-studied loci that included D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and sex chromosome Amelogenin were chosen. The sequencing process of STR was outsourced to Jabatan Kimia Malaysia, Johor branch. The STR profile obtained was analyzed using GeneMapper software (version 3.2) and the observed polymorphisms were recorded.

RESULTS

Cell Culture

The urinary bladder cancer tissue used in this study survived over 15 passages. The cells of the newly established cell line demonstrated spindle-shaped morphology with clearly defined margins (Figure 1) and exhibited good viability when re-cultured from cryopreserved stocks. PCR assay was performed to detect the presence of *Mycoplasma sp.* and our result revealed that such contamination was not detected (Figure 2).

Growth Characteristic

It was found that the newly established USM-BC-1 cell line had 44.8 hours of PDT as well as 67 % and 2 % of attachment efficiency and plating efficiency, respectively (Table 1) and the growth curve of USM-BC-1 is presented in Figure 3.

Immunocytochemistry

Our finding reveals that the USM-BC-1 was positively stained (brown) for cytokeratin 7 (Figure 4) while negatively stained (blue) for desmin (Figure 5). These results confirmed that the cells were of epithelial origin and neither fibroblast nor smooth muscle cells were present in the USM-BC-1 cell line.

Western blot

Our study on the Ki-67 (345 kDa), VEGF (42 kDa) and p21 (21 kDa) indicated that those proteins were expressed in our USM-BC-1 cell line although the levels of expression varied; antigen Ki-67 being highly expressed while the expression of VEGF and p21 remained low (Figure 6-8).

STR analysis

The STR profile for USM-BC-1 cell line was found to be similar with that of the donor's urinary bladder cancer tissue sampled in this study, indicating 96% of similarity (Table 2). It was found that the sequence of STR differed at one locus (D2S1338) in which allele 19 was evidently replaced by allele 26.

DISCUSSION

It is pertinent to mention here that the establishment of human urinary bladder cancer cell lines is of importance in investigating the possibility of producing monoclonal antibodies [10], cytogenetic changes [4], identification and isolation of oncogenes [11] as well as developing effective anti-cancer drugs [3] hence, requiring the use of reliable established cell lines. It has been indicated in the ATCC database that most of the currently used human urinary bladder cancer cell lines were established from Caucasian populations while its use has been generalized over various populations throughout the world [12-14] although neoplastic cells behavior attributable to differences in ethnicities has been reported [7]. Furthermore, review of the literature reveals that the majority of the currently used human urinary bladder cancer cell lines were established within the last three to four decades [15], perhaps in environmental conditions that were different from the current highly industrialized world [7] as well as insufficiently characterized [16]. Therefore, in this context the establishment of the USM-BC-1 cell line for studying the various aspects of cancer acquires significance.

In this study, it was found that the cancer cells in the newly established USM-BC-1 cell line were of spindle-shaped in morphology with clearly defined margins, which was in concurrence with the morphology of the previously reported established cell lines of human urinary bladder cancer [7, 17]. However, being cancerous, observing phenotypic instability that includes morphological changes is common in cultured cell lines [16, 18] which ranged from small round morphology [19], spindle-shaped morphology [17], to heterogeneity of polygonal and spindled-shaped morphology [16].

Similar studies that involved establishing new cancer cell lines indicated that PDT ranged between 20-90 hours [7, 16, 19, 20] although specific standard for assigning the efficiency of the PDT remains lacking. The variations in PDT maybe attributable to various factors that include the types of media, nutrients, pH, temperature, dissolved oxygen concentration and environmental conditions [21]. Moreover, for optimum utilization of the newly established cancer cell lines, Langdon (2004) suggested that the attachment efficiency of such cell lines should not be lower than 60%. While considerably higher percentages of plating efficiency for the well established cancer cell lines have been reported in the ATCC database, many previous studies attempted at establishing new cancer cell lines reported lower percentages of plating efficiency that ranged between 0 - 4% [16, 20, 22-24], signifying the needs for using larger amount of seeding cells for optimum analysis. In this study, the PDT, attachment efficiency as well as plating efficiency were 44.8 hours, 67% and 2% respectively, fell well within the prescribed values. Therefore, the USM-BC-1 cell line established in this study proves to be useful.

It has been shown that morphology of the transitional cell carcinoma (TCC) of urinary bladder is composed of epithelial cells [25]. The fact that cytokeratin 7 has been widely used as one of the reliable markers in differential diagnosis of carcinoma of epithelial origin [26, 27], its detection for ascertaining the identity of the urinary bladder TCC in the newly established USM-BC-1 cell line acquires significance. In this study, cytokeratin 7 was found to be positively stained in the newly established USM-BC-1 cell line, indicating the possible presence of urinary bladder TCC. However, since the morphology of the cells in the USM-BC-1 cell line was found to be spindle-like in shape which was similar to the morphology of fibroblast, the detection of desmin was deemed necessary for confirming the identity of the cells. Desmin has been reported as one of the potential markers for detecting the presence of fibroblast and muscle cells [28]. Our results revealed that desmin was negatively stained in the USM-BC-1 cell line, a positive indication for the presence of urinary bladder TCC alone.

The established USM-BC-1 cell line was further characterized by evaluating the expression of three important biomarkers for cancer namely Ki-67, VEGF and p21 proteins. Our result revealed that the Ki-67 protein was highly expressed while the expression of VEGF and p21 proteins remained low in the USM-BC-1 cell line. It has been reported in previous studies that in cancer cells, high expression of Ki-67 protein is common [29, 30] since this protein is responsible for proliferation of cancer cells [31], correlating with higher grade of cancer and poor prognosis [29, 32]. In addition, the expression of VEGF protein that plays an important role in angiogenesis, a factor responsible for sustaining the growth of cancer has been reported to be expressed in many cancer cells by previous researchers [33-35]. However, in our newly established USM-BC-1 cell line such expression was evidently low, indicating the possible role of other angiostimulatory proteins such as as midkine, tissue factor (TF), hepatocyte growth factor (HGF) and angiogenic fragments of hyaluronic acid in promoting angiogenesis [36-38]. This aspect deserves further study. Being a cell cycle regulator gene, p21 is usually expressed in non-cancerous cells and its alteration or loss of expression would lead to unregulated cell growth and formation of cancer cells [39]. Therefore, it appears that the

cancer biomarkers selected in this study further confirm the characterization of the USM-BC-1 as cancer cell line, which may have an applied value for further research.

It has been widely reported that cross-contamination and misidentification of the established cell lines would lead to scientific misrepresentation and possibly erroneous interpretation of results [40]. Alarmingly, 18% of the 252 cell lines submitted to the German Cell Line Bank [41] as well as 36% of the cell lines submitted to the National Testing Services were found to be cross-contaminated [42]. Therefore, it has been cautioned by many researchers [40, 43-45] that the authentication of the newly established cell lines is of great importance and many techniques such as enzyme polymorphisms [46], karyotyping [47], HLA typing [48], immunophenotypic and immunocytochemical analysis [27] and DNA fingerprinting [49] have been suggested. However, due to the lack of standard references, the application of such techniques for authentication purposes maybe limited. In addition, alteration of the DNA fingerprinting profiles for cancer cell lines over a period of time has been reported, restricting its use as stable identifiers for a particular cell line [43]. The fact that the standard references are available for STR analysis, the technique has acquired considerable popularity worldwide as a means for authentication of the newly established cell lines [40]. In this study, it was found that the STR profile of the USM-BC-1 cell line performed at the 15^{th} passage demonstrated 96% similarity with the sampled donor's urinary bladder cancer tissue; the difference being allele 19 at D2S1338 locus was evidently replaced by allele 26. For ensuring the practical value of the newly established cell line for research purposes, it has been suggested that the STR profile needs to be 80% similar to that of the donor's tissue [40, 50]. Variations in the STR profiles of established cell lines when compared with the tissues of the donors maybe attributable to genetic instability of cancer cells [7] probably due to the loss of heterozygosity and microsatellite instability [51, 52]. Since the STR profile for the newly established USM-BC-1 cell line was found to be 96% similar to the donor's tissue, fell well within the prescribed range of similarity, the USM-BC-1 cell line proved to be authentic.

CONCLUSION

In conclusion, the newly established and well-characterized USM-BC-1 cell line would provide an ideal in vitro model for Malaysian population that may serve as a valuable tool for developing future diagnostic and therapeutic targets. This present study utilized a specimen of urinary bladder cancer tissue obtained from a Malay patient using a single medium of RPMI-1640. Here, the limitations are; the establishment of such cell line may not be representing the genetic variations that maybe present among the different races in Malaysia namely Malays, Chinese, Indians and others, as well as the efficiency of establishing the USM-BC-1 cell line may not be comparable with other cell lines since only one medium was used for culturing. Therefore, further researches involving a larger number of samples obtained from various races in Malaysia, the use of various media for culturing, as well as investigation on the single nucleotide polymorphisms between the newly established cell line with the available cell lines in the market, merit further research. Having said that, the USM-BC-1 cell line established in this study for urinary bladder cancer research would still be considered useful since this was the first attempt at establishing such cell line in Malaysia.

COMPETING INTERESTS

We declare that we have neither financial competing nor non-financial competing interests.

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REFERENCES

[1] Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., and Thun, M.J. (2008) Cancer statistics, 2008. CA: a cancer journal for clinicians. 58, pp. 71-96.

[2] Zainal Ariffin, O., and Nor Saleha, I.T. (2011) National Cancer Registry Report 2007. Ministry of Health, Malaysia.

[3] Ikemoto, S., Sugimura, K., Yoshida, N., Yasumoto, R., Wada, S., Yamamoto, K., and Kishimoto, T. (2000) Antitumor effects of Scutellariae radix and its components baicalein, baicalin, and wogonin on bladder cancer cell lines. Urology. 55, pp. 951-955.

[4] Williams, S.V., Sibley, K.D., Davies, A.M., Nishiyama, H., Hornigold, N., Coulter, J., Kennedy, W.J., Skilleter, A., Habuchi, T., and Knowles, M.A. (2002). Molecular genetic analysis of chromosome 9 candidate tumor-suppressor loci in bladder cancer cell lines. Genes, Chromosomes and Cancer. 34, pp. 86-96.

[5] Shen, S.S., Smith, C.L., Hsieh, J.T., Yu, J., Kim, I.Y., Jian, W., Sonpavde, G., Ayala, G.E., Younes, M., and Lerner, S.P. (2006) Expression of estrogen receptors-α and -ß in bladder cancer cell lines and human bladder tumor tissue. Cancer. 106, pp. 2610-2616.

[6] Hatina, J., Huckenbeck, W., Rieder, H., Seifert, H.H., and Schulz, W.A. (2008) Bladder carcinoma cell lines as models of the pathobiology of bladder cancer. Review of the literature and establishment of a new progression series. Der Urologe. 47, pp. 724.

[7] Piantino, C.B., Sousa-Canavez, J.M., Srougi, V., Salvadori, F., Kato, R., Ayres, P.P.R., Srougi, M., Camara-Lopes, L.H., Gattàs, G.J.F., and Fridman, C. (2009) Establishment and characterization of human bladder cancer cell lines BexBra1, BexBra2, and BexBra4. In Vitro Cellular & Developmental Biology-Animal. 46, pp. 131-139.

[8] Drexler, H.G., and Uphoff, C.C. (2002) Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology. 39, pp. 75-90.

[9] Walker, J. M. (1996) The protein protocols handbook. Humana PressInc, New Jersey.

[10]Young, D.A., Prout, G.R., and Lin, C.W. (1985) Production and characterization of mouse monoclonal antibodies to human bladder tumor-associated antigens. Cancer Research. 45, pp. 4439-4446.

[11] Cooper, G.M. (1982) Cellular transforming genes. Science. 217, pp. 801-806.

[12] Hasegawa, S., Abe, T., Naito, S., Kotoh, S., Kumazawa, J., Hipfner, D.R., Deeley, R.G., Cole, S.P., and Kuwano, M. (1995) Expression of multidrug resistance-associated protein (MRP), MDR1 and DNA topoisomerase II in human multidrug-resistant bladder cancer cell lines. British Journal of Cancer. 71, pp. 907.

[13] Ribeiro Filho, L.A., Franks, J., Sasaki, M., Shiina, H., Li, L.C., Nojima, D., Arap, S., Carroll, P., Enokida, H., and Nakagawa, M. (2002) CpG hypermethylation of promoter region and inactivation of E-cadherin gene in human bladder cancer. Molecular Carcinogenesis. 34, pp. 187-198.

[14] Tian, B., Wang, Z., Zhao, Y., Wang, D., Li, Y., Ma, L., Li, X., Li, J., Xiao, N., and Tian, J. (2008) Effects of curcumin on bladder cancer cells and development of urothelial tumors in a rat bladder carcinogenesis model. Cancer Letters. 264, pp. 299-308.

[15] Knuechel, R., and Masters, J.R.W. (1999) Bladder cancer. Human Cell Culture. 1, pp. 213-230.

[16] Morita, T., Shimohara, N., Honma, M., and Tokue, A. (1995) Establishment and characterization of a new cell line from human bladder cancer (JMSU1). Urological Research. 23, pp. 143-149.

[17] Ozu, C., Takeuchi, H., Matsushita, K., Horiguchi, Y., Namiki, K., Yoshioka, K., Nagai, T., and Tachibana, M. (2009) Establishment and characterization of a new squamous cell carcinoma cell line, TMUU-8, derived from human bladder cancer. Human Cell. 22, pp. 25-29.

[18] Duesberg, P., Rausch, C., Rasnick, D., and Hehlmann, R. (1998) Genetic instability of cancer cells is proportional to their degree of aneuploidy. Proceedings of the National Academy of Sciences 95, pp. 13692-13697.

[19] Kakuya, T., Yamada, T., Yokokawa, M., and Ueda, T. (1983) Establishment of cell strains from human urothelial carcinoma and their morphological

characterization. In Vitro Cellular & Developmental Biology-Plant. 19, pp. 591-599.

[20]Offner, F.A., Ott, G., Povey, S., Knuechel, R., Preisler, V., Fuezesi, L., Klosterhalfen, B., Ruebben, H., Hofstaedter, F., and Kirkpatrick, C.J. (1991) Characterization of the new bladder cancer cell line HOK-I: Expression of transitional, squamous and glandular differentiation patterns. International Journal of Cancer. 49, pp. 122-128.

[21] Langdon, S.P. (2004) Cancer cell culture: methods and protocols. Humana press, New Jersey.

[22] Lin, C.W., Lin, J.C., and Prout, G.R. Establishment and characterization of four human bladder tumor cell lines and sublines with different degrees of malignancy. (1985) Cancer Research. 45, pp. 5070-5079.

[23] Grippo, M.C., Penteado, P.F., Carelli, E.F., Cruz-H□fling, M.A., and Verinaud, L. (2001) Establishment and partial characterization of a continuous human malignant glioma cell line: NG97. Cellular and Molecular Neurobiology. 21, pp. 421-428.

[24]Wang, J., Wang, X., Jiang, S., Lin, P., Zhang, J., Wu, Y., Xiong, Z., Ren, J.J., and Yang, H. (2007) Establishment of a new human glioblastoma multiforme cell line (WJ1) and its partial characterization. Cellular and Molecular Neurobiology. 27, pp. 831-843.

[25] Murphy, W.M., Grignon, D.J., and Perlman, E.J. (2004) AFIP atlas of tumor pathology, series 4. Tumors of the Kidney, Bladder, and Related Urinary Structures. American Registry of Pathology, Washington.

[26] Chu, P., Wu, E., and Weiss, L.M. (2000) Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Modern Pathology. 13, pp. 962-972.

[27] Quentmeier, H., Osborn, M., Reinhardt, J., Zaborski, M., and Drexler, H.G. (2001) Immunocytochemical analysis of cell lines derived from solid tumors. Journal of Histochemistry & Cytochemistry. 49, pp. 1369-1378.

[28] Kalluri, R., and Zeisberg, M. (2006) Fibroblasts in cancer. Nature Reviews Cancer 6, pp. 392-401.

[29]Okamura, K., Miyake, K., Koshikawa, T., and Asai, J. (1990) Growth fractions of transitional cell carcinomas of the bladder defined by the monoclonal antibody Ki-67. The Journal of Urology. 144, pp. 875.

[30] Fontana, D., Bellina, M., Gubetta, L., Fasolis, G., Rolle, L., Scoffone, C., Porpiglia, F., Colombo, M., Tarabuzzi, R., and Leonardo, E. (1992) Monoclonal antibody Ki-67 in the study of the proliferative activity of bladder carcinoma. The Journal of Urology. 148, pp. 1149.

[31] Scholzen, T., and Gerdes, J. (2000) The Ki-67 protein: from the known and the unknown. Journal of Cellular Physiology. 182, pp. 311-322.

[32] Norming, U., Tribukait, B., Nyman, C.R., Nilsson, B., and Wang, N. (1992) Prognostic significance of mucosal aneuploidy in stage Ta/T1 grade 3 carcinoma of the bladder. The Journal of Urology. 148, pp. 1420.

[33]Crew, J.P., O'Brien, T., Bradburn, M., Fuggle, S., Bicknell, R., Cranston, D., and Harris, A.L. (1997) Vascular endothelial growth factor is a predictor of relapse and stage progression in superficial bladder cancer. Cancer Research. 57, pp. 5281-5285.

[34]Campbell, S.C., Volpert, O.V., Ivanovich, M., and Bouck, N.P. (1998) Molecular mediators of angiogenesis in bladder cancer. Cancer Research. 58, pp. 1298-1304.

[35]Yang, C.C., Chu, K.C., and Yeh, W.M. (2004) The expression of vascular endothelial growth factor in transitional cell carcinoma of urinary bladder is correlated with cancer progression. In Urologic Oncology: Seminars and Original Investigations. 22, pp. 1-6.

[36] Joseph, A., Weiss, G.H., Jin, L., Fuchs, A., Chowdhury, S., O'Shaugnessy, P., Goldberg, I.D., and Rosen, E.M. (1995) Expression of scatter factor in human bladder carcinoma. Journal of the National Cancer Institute. 87, pp. 372-377.

[37]O'Brien, T., Cranston, D., Fuggle, S., Bicknell, R., and Harris, A.L. (1995) Different angiogenic pathways characterize superficial and invasive bladder cancer. Cancer Research. 55, pp. 510-513.

[38] Lokeshwar, V.B., Obek, C., Soloway, M.S., and Block, N.L. (1997) Tumor-associated hyaluronic acid: a new sensitive and specific urine marker for bladder cancer. Cancer Research. 57, pp. 773-777.

[39]Gartel, A.L., Serfas, M.S., and Tyner, A.L. (1996) p21-negative regulator of the cell cycle. Proceedings of the Society for Experimental Biology and Medicine. New York, pp. 138-149.

[40] Masters, J.R., Thomson, J.A., Daly-Burns, B., Reid, Y.A., Dirks, W.G., Packer, P., Toji, L.H., Ohno, T., Tanabe, H., and Arlett, C.F. (2001) Short tandem repeat profiling provides an international reference standard for human cell lines. Proceedings of the National Academy of Sciences. Seattle, pp. 8012-8017.

[41]MacLeod, R.A.F., Dirks, W.G., Matsuo, Y., Kaufmann, M., Milch, H., and Drexler, H.G. (1999) Widespread intraspecies cross-contamination of human tumor cell lines arising at source. International Journal of Cancer. 83, pp. 555-563.

[42]Markovic, O., and Markovic, N. (1998) Cell cross-contamination in cell cultures: the silent and neglected danger. In Vitro Cellular & Developmental Biology-Animal. 34, pp. 1-8.

[43] Parson, W., Kirchebner, R., Muhlmann, R., Renner, K., Kofler, A., Schmidt, S., and Kofler, R. (2005) Cancer cell line identification by short tandem repeat profiling: power and limitations. The FASEB Journal. 19, pp. 434-436.

[44] Azari, S., Ahmadi, N., Tehrani, M.J., and Shokri, F. (2007) Profiling and authentication of human cell lines using short tandem repeat (STR) loci: Report from the National Cell Bank of Iran. Biologicals. 35, pp. 195-202.

[45] Chiong, E., Dadbin, A., Harris, L.D., Sabichi, A.L., and Grossman, H.B. (2009) The use of short tandem repeat profiling to characterize human bladder cancer cell lines. The Journal of Urology. 181, pp. 2737-2748.

[46]Nims, R.W., Shoemaker, A.P., Bauernschub, M.A., Rec, L.J., and Harbell, J.W. (1998) Sensitivity of isoenzyme analysis for the detection of interspecies cell line cross-contamination. In Vitro Cellular & Developmental Biology-Animal. 34, pp. 35-39.

[47] Rush, L.J., Heinonen, K., Mrózek, K., Wolf, B.J., Abdel-Rahman, M., Szymanska, J., Peltomäki, P., Kapadia, F., Bloomfield, C.D., and Caligiuri, M.A. (2002) Comprehensive cytogenetic and molecular genetic characterization of the TI-1 acute myeloid leukemia cell line reveals cross-contamination with K-562 cell line. Blood. 99, pp. 1874-1876.

[48] O'Toole, C.M., Povey, S., Hepburn, P., and Franks, L.M. (1983) Identity of some human bladder cancer cell lines. Nature. 301, pp. 429-430.

[49] Gilbert, D.A., Reid, Y.A., Gail, M.H., Pee, D., White, C., Hay, R.J., and O'Brien, S.J. (1990) Application of DNA fingerprints for cell-line individualization. American Journal of Human Genetics. 47, pp. 499-507.

[50] Lorenzi, P.L., Reinhold, W.C., Varma, S., Hutchinson, A.A., Pommier, Y., Chanock, S.J., and Weinstein, J.N. (2009) DNA fingerprinting of the NCI-60 cell line panel. Molecular Cancer Therapeutics. 8, pp. 713-724.

[51] Poetsch, M., Petersmann, A., Woenckhaus, C., Protzel, C., Dittberner, T., Lignitz, E., and Kleist, B. (2004) Evaluation of allelic alterations in short tandem

repeats in different kinds of solid tumors-possible pitfalls in forensic casework. Forensic Science International. 145, pp. 1-6.

FIGURE/TABLE CAPTION

Figure 1 The USM-BC-1 cell line at time of confluency indicating spindle-shaped morphology cells.

Figure 2 Electrophoresis of PCR amplification products on 2% agarose gel for Mycoplasma sp investigation. Absence of Mycoplasma sp. contaminated in the USM-BC-1 cell line on gel electrophoresis (B). The presence of a 270 bp fragment would positively indicate contamination by Mycoplasma sp while a 603 bp fragment represented the internal standard.

Figure 3 Growth curve of USM-BC-1 cells.

Figure 4 Immunocytochemistry staining of the USM-BC-1 cell line at the 10th passage with cytokeratin 7. Immunopositive staining of Cytokeratin 7 for (A) USM-BC-1 and (B) HeLa as the positive control. Immunonegative staining of cytokeratin 7 in negative control with the omission of primary antibody (C).

Figure 5 Immunocytochemistry staining of the USM-BC-1 cell line at the 10th passage with desmin. (A) Absence of desmin in USM-BC-1 and (B) negative control with the omission of primary antibody while desmin was observed in in SJRH30 positive control.

Figure 6 Western blot analysis of the Ki-67 protein expression. (A) Positive control (Hela whole cell lysate), **(B)** USM-BC-1 whole cell lysate and **(C)** Negative control. High expression of Ki-67 protein at 345 kDa was observed in the USM-BC-1 whole cell lysate **(B)** compared to the positive control HeLa whole cell lysate **(A)**.

Figure 7 Western blot analysis of the VEGF protein expression. **(A)** Negative control, **(B)** USM-BC-1 whole cell lysate and **(C)** Positive control. Low expression of VEGF protein at 42 kDa was observed in the USM-BC-1 whole cell lysate **(B)**.

Figure 8 Western blot analysis of the p21 protein expression. (A) Positive control (MDA-MB-231 whole cell lysate), **(B)** Negative control and **(C)** USM-BC-1 whole cell lysate. Low expression of p21 protein at 21 kDa was observed in the USM-BC-1 whole cell lysate **(C)**.

Table 1. Attachment efficiency and plating efficiency of USM-BC-1 cell line.

Table 2. The STR profile of donor's tumor tissue versus USM-BC-1 cell line.
[a]Indicates polymorphism observed in the USM-BC-1 cell line when compared with the sequence of STR in the donor's cancer tissue.

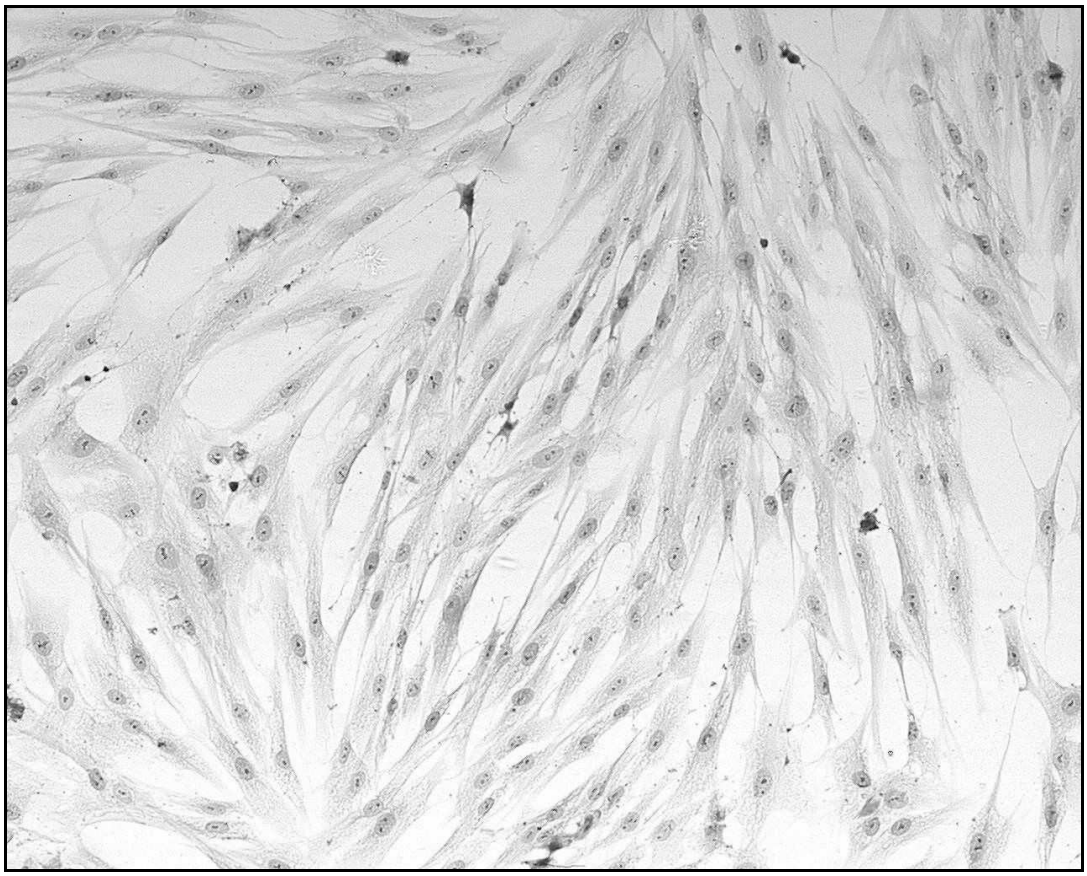

Figure 1

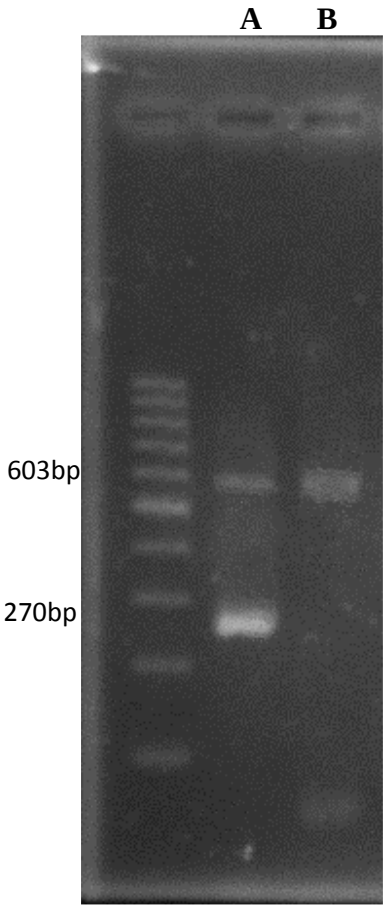

Figure 2

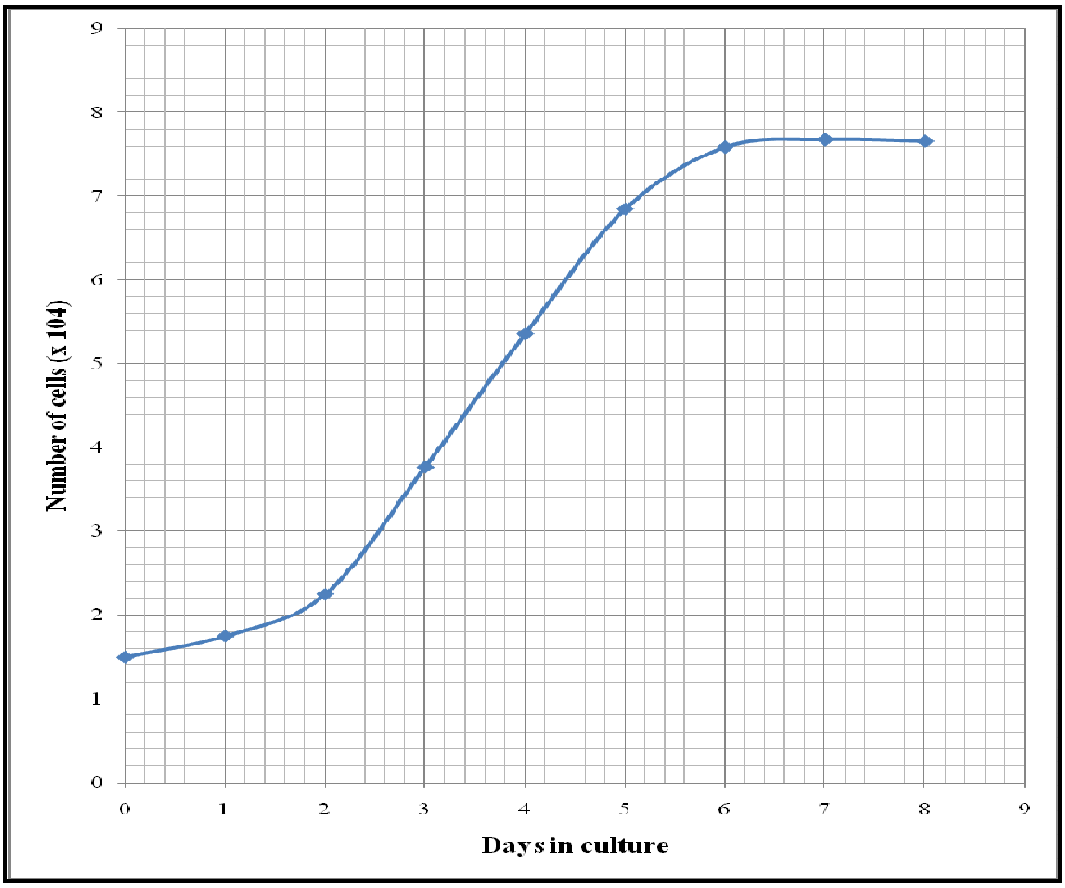

Figure 3

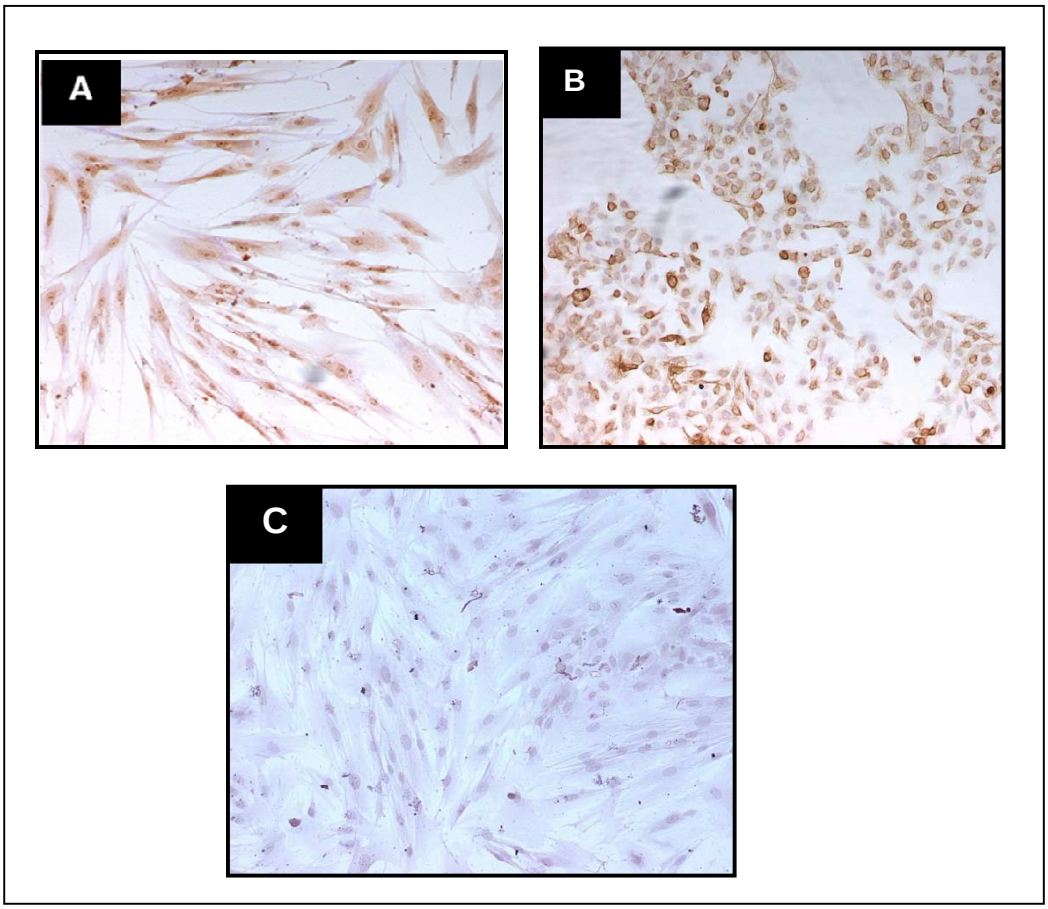

Figure 4

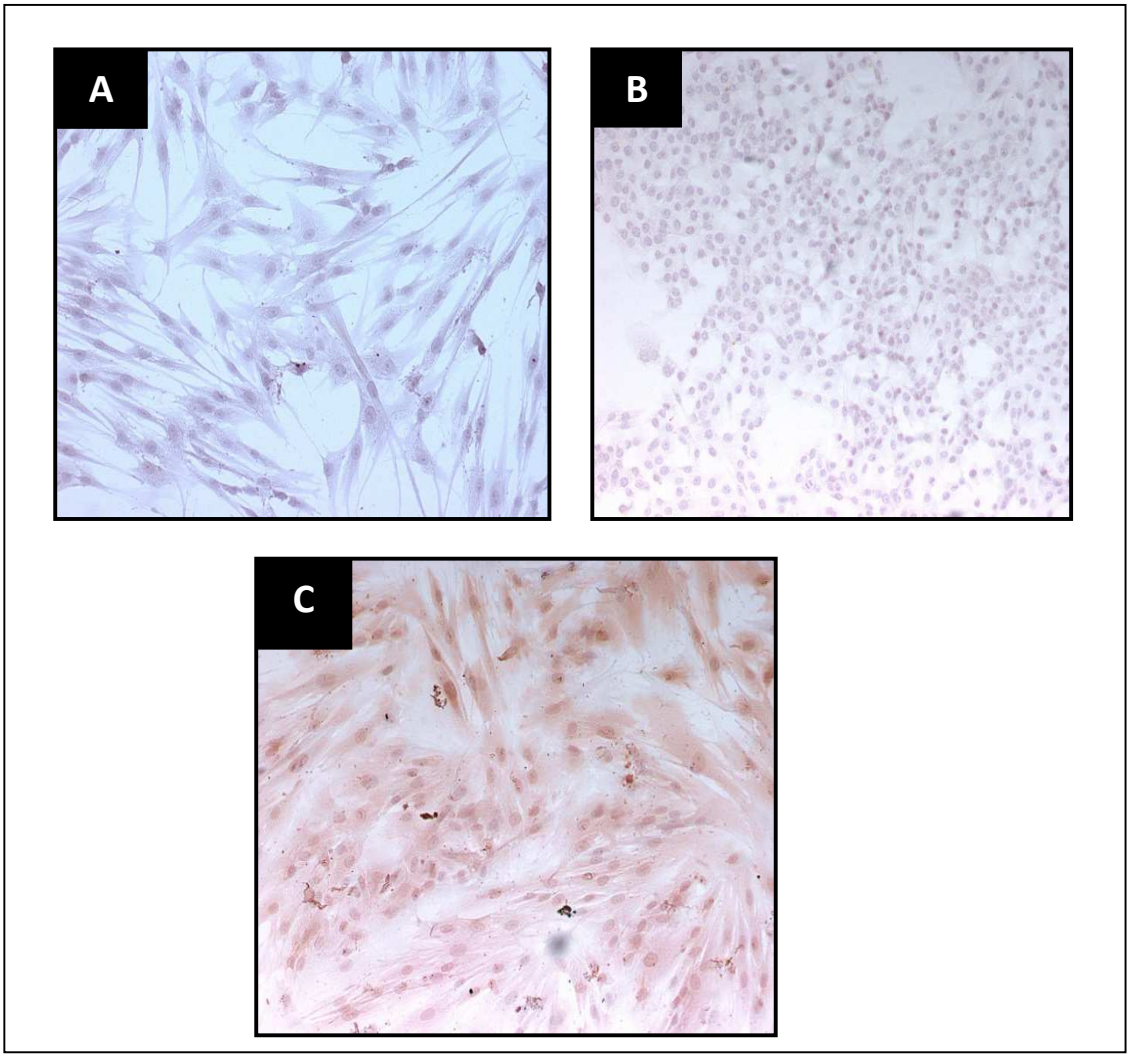

Figure 5

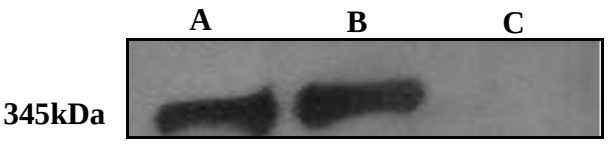

Figure 6

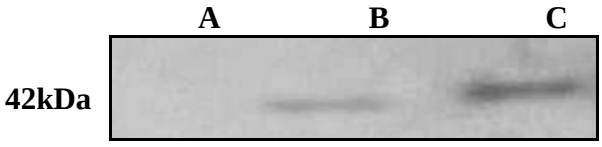

Figure 7

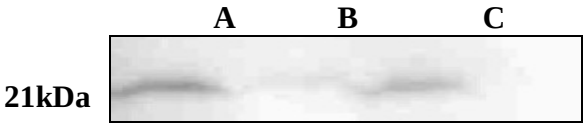

Figure 8

Initial Count	Count of cells after 12 hours	Attachment efficiency
3.5×10^4	2.33×10^4	67 %
Initial Count	Count of macrocolonies after 10 days	Plating efficiency
100	2	2 %

Table 1

Locus Designation	Donor's Tumor Tissue	USM-BC-1
D8S1179	13, 15	13, 15
D21S11	31, 31.2	31, 31.2
D7S820	10, 11	10, 11
CSF1PO	11, 12	11, 12
D3S1358	16, 17	16, 17
TH01	9.3, 10	9.3, 10
D13S317	11, 11	11, 11
D16S539	10, 11	10, 11
[a]D2S1338	19, 19	19, 26
D19S433	15.2, 16	15.2, 16
vWA	15, 17	15, 17
TPOX	9, 11	9, 11
D18S51	17, 18	17, 18
Amelogenin	X,Y	X,Y
D5S818	10, 12	10, 12
FGA	22, 25	22, 25

Table 2