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Animal Posters

A-2000

Mechanisms of Resistance to Oxidative Stress in Leukemia Cells. J. H. WEISBURG and M. Vigodner. Stern College for Women, Department of Biology, 245 Lexington Avenue, New York, NY 10016. Email: weisburg@yu.edu

Cellular resistance to cytotoxic agents has been of great importance for the treatment of cancer. Many of these agents generate reactive oxygen species (ROS), and cross-resistance to ROS has been observed in multi-drug resistance (MDR). We compared mechanisms of ROS resistance in an in vitro model of MDR cells selected in the presence of vincristine (RV+) with cells selected from direct exposure to ROS via hydrogen peroxide (RTP). From this comparison, we determined if ROS alone are sufficient to confer resistance to a ROS-sensitive cell line, and distinguished ROS toxicity from broader effects that might underlie cross-resistance to MDR. Although both lines were highly resistant in comparison to parental HL60 cells, RV+ and RTP cells (ten- and twenty-fold resistance over HL60, respectively) differed widely in their dependence on key ROS-mitigating pathways, gluthatione reductase(GR) and catalase. Pretreatment of the cells with buthionine sulphoximine(BSO) reverted H_2O_2 resistance of RV+ cells to the level of HL60. Whereas, the BSO treated RTP cells were only partially affected, suggesting RV+, and not RTP, were exclusively dependent on the GR pathway. Consistent with this RTP and HL60 had lower baseline glutathione (GSH) levels that decreased upon H_2O_2 exposure, while RV+ cells had substantially higher baseline GSH that increased under the same conditions. Alluding to an RTP dependence on the catalase pathway, we demonstrated that HL60 and RTP cells had elevated catalase activity compared to the RV+. Upon treatment with H_2O_2, nuclear erythroid related factor 2 (Nrf2), a transcription factor known to regulate both GR and catalase, translocated to the nucleus with kinetics correlative to ROS resistance. Nrf2 translocation kinetics may act as a key determinate to ROS resistance, irrespective of the ROS-mitigating pathway. Ultimately, we demonstrated the broad range of cellular mechanisms that can arise from either direct exposure to ROS, or indirect exposure from the chemotherapeutic metabolites. These observations may have impact on the clinical treatment of MDR cells.

A-2001

Isolation, Culture, and Characterization of a Fibroblast Cell Line from Skin Samples of Saanen Breed of Goats. MAHIPAL SINGH[1] and Anil K. Sharma[2]. [1]Animal Science Division, Fort Valley State University, Fort Valley, GA 31030 and [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55901. Email: singhm@fvsu.edu

Establishing quality fibroblast cell lines from small ruminants is important for several reasons including their use as feeders, for developing iPS cells, in grafts, for manipulating genome, and to study fibroblast function in in-vitro cultures. In this study a fibroblast cell line (named as GSF289) was established from ear skin explants of normal healthy dairy goats, of Saanen breed. Liquid nitrogen stocks of the frozen cells had a viability rate of 96.2% in in-vitro cultures. These cells were morphologically indistinguishable from the cell stocks prior to freezing. Analysis of the growth of a fifth passage culture revealed an 'S' shaped growth curve with a population doubling time of 25 hrs. The cell line was found negative for microbial, fungal and

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mycoplasma contamination. The GSF289 cell line was successfully transfected with pcDNA3.1/NT-GFP plasmid vector containing green fluorescent protein (GFP) gene under human cytomegalovirus (CMV) promoter. The efficiency of transfection, as measured by flow cytometry, was 14.5% after 4 d of culture. The cytogenetic analysis performed on 29 G-banded metaphase cells revealed that the cell line has a normal male goat karyotype consisting of 58 autosomes and two XY sex chromosomes. These results suggest that GSF289 cell line with a normal karyotype, having a high rate of proliferation, and its ability to be easily transfected with plasmid DNA vectors is an important tool and can be used for genetic manipulation of small ruminants as well as to study molecular mechanisms that regulate fibroblast function.

A-2002

Translation Initiation Factor eIF4G-1 Function During Apoptosis in Breast Cancer Cells. W. K. CROSSON, A. R. Blackwell[1,3], B. D. Keiper[2], and M. A. Farwell[1]. [1]Department of Biology, East Carolina University; [2]Department of Biochemistry and Molecular Biology, Brody School of Medicine; and [3]PPD, Inc., Research Triangle Park, NC. Email: crossonw09@students.ecu.edu

Cell proliferation and apoptosis are linked to specific gene expression events regulated by protein synthesis, which begins with the joining of various eukaryotic initiation factors (eIF) to mRNA and ribosomes to initiate translation. eIF4G-1 catalyzes two types of translation initiation. Cap-dependent translation requires eIF4E to bind a 5'-methylated mRNA cap and eIF4G-1 to facilitate recruitment and promotes translation of cell cycle and growth-related proteins. Cap-independent translation initiates internally through internal ribosome entry sites (IRES) and promotes translation of apoptotic mRNAs such as Apaf-1. We find that 5 isoforms and 8 variants of eIF4G-1 exist, each potentially forming a translation complex with differing activities. We hypothesized that the representation of eIF4G isoforms will vary, as will the predominant mode of translation initiation, in aggressive, tumor-forming breast cell lines vs. non-tumor-forming lines. However, when identification of eIF4G-1 isoform representation was determined in three breast carcinoma cell lines and one non-tumorigenic breast epithelium cell line, no such systematic increase or decrease of individual isoforms was found. We have now developed a novel dual fluorescence reporter containing the Apaf-1 IRES, or a viral IRES to assay the propensity of these cells toward cap-independent translation in vivo. Our preliminary results confirm the ability of this assay to measure the ratio of cap-dependent versus cap-independent initiation in single live cells as demonstrated by blue fluorescence or green fluorescence, respectively. We will next deplete eIF4G-1 isoforms in these cell lines, treat them with apoptotic drugs, and evaluate temporal shifts in their balance of cap-dependent to cap-independent synthesis, as well as specific utilization of the apoptotic IRES. By establishing the role of eIF4G-1 isoforms in pro-apoptotic protein synthesis, it may be possible to push a cell from proliferation to apoptosis by targeting certain isoforms.

A-2003

Determing Early Signaling Response to Environmental Toxicants Using Novel Proteomic Approaches. B. L. UPHAM[1], P. Babica[1], Joon-Suk Park[1], D. A. Whitten[2], and C. G. Wilkerson[2]. [1]Department of Pediatrics & Human Development and the National Food Safety & Toxicology Center and [2]RTSF Proteomic Core, Michigan State University, East Lansing, MI. Email: upham@msu.edu

Identifying early signaling responses of mammalian cells to environmental contaminants is vital in determining the underlying mechanisms of toxicity. We developed two novel strategies that capitalize on the latest technologies of proteomics to investigate changes in the general proteome and phospho-specific proteome in a rat liver oval-like epithelial cell line (WB-F344). Each strategy used "Stable Isotopes Labeling with Amino Acids in Cell Culture (SILAC)" that allowed for an accurate quantification of the proteins. Strategy-1 used 2-dimensional ZOOM® isoelectric fractionation (2D-ZOOM) to separate proteins that were extracted from the membrane and cytosolic fraction of the control vs. toxicant to identify any proteomic changes. This data was then used to identify the proteins to be assessed by mass spectrometry on a 2 d-ZOOM gel loaded with proteins from the SILAC experiment (same treatment except that the toxicant treated cells were grown on SILAC medium with proteins extracted and combined from the control vs. toxicant and loaded onto one gel). The second approach used TiO_2 enrichment of phosphopeptides from the SILAC experiment and then used multidimensional separation coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS) for identifying changes in the phospho-proteome. We used an *active* (inhibits gap junction, activate MAPKs and induce arachidonate release) and an *inactive* isomer of methylanthracene to identify proteomic changes. The few phosphoproteins identified in the first more general approach were also

identified in the second approach which resulted in the identification of hundreds of phosphoproteins. Support: NIEHS grant #R01 ES013268-01A2 to upham.

A-2004

Decrease in Intracellular Glutathione and Induction Apoptosis in HSC-2 Carcinoma Cells from the Human Oral Cavity Due to Pomegranate Juice Extract. Y. A. Hirth, H. L. Zuckerbraun, A. G. Schuck, and J. H. WEISBURG. Stern College for Women, Department of Biology, 245 Lexington Avenue, New York, NY 10016. Email: weisburg@yu.edu

Previous work in our laboratory showed that pomegranate extract (PE) behaved as a prooxidant, generating hydrogen peroxide (H_2O_2) in cell culture medium and inducing oxidative stress in the target cells. A hallmark of oxidative stress is the lessening of the intracellular level of reduced glutathione (GSH) in cells exposed to an oxidant. GSH, a thiol-containing tripeptide, is the main intracellular antioxidant in the cell's repertoire against oxidative defense. HSC-2 cells treated with increasing concentrations of PE demonstrated a progressively decreasing content of intracellular GSH. Depletion of intracellular GSH upon exposure to PE was confirmed by fluorescent staining of intracellular thiols using Cell-Tracker™ Green CMFDA 5-chloromethylfluorescein diacetate. The intensity of the green fluorescence was a function of the concentration of intracellular thiol-containing molecules. Control cells not exposed to PE stained bright green, whereas little fluorescence was noted in HSC-2 cells exposed to PE. Oxidative stress is a known inducer apoptosis. Flow cytometric analyses of HSC-2 cells untreated and treated with PE showed that as the concentration of PE increased, the number of viable cells decreased and the numbers both of apoptotic and of dead cells increased.

A-2005

Evaluation and Identification of Reliable Reference Genes for Pharmogenomics, Toxicogenomics and Small RNA Expression Analysis in Human Cell Line MCF-7. DONGLIANG CHEN and Baohong Zhang. Department of Biology, East Carolina University, Greenville, NC 27858. Email: chend09@students.ecu.edu

Pharmogenomics, toxicogenomics and small RNA expression analysis are three of the most active research topics. All of these investigations are based on gene expression analysis which requires reference genes to reduce the variations derived from different amount of starting materials and different efficiency of RNA extraction and cDNA synthesis. Although in previous studies, selection of reliable reference genes has been conducted in several animals and plants, no research has been focused on pharmacological research and very few researches were involved in toxicological context. More interestingly, few studies have been performed to identify small nucleolar RNAs as reference genes, although some of them are frequently used in expression analysis. In this study, we employed quantitative real-time PCR (qRT-PCR) to evaluate and to screen the reliable reference genes for pharmogenomics and toxicogenomics studies as well as for small RNA expression analysis. MCF-7 breast cancer cells were chosen as a model in this study. We tested the transcriptional expression of five protein-coding genes as well as five non-coding genes in human breast cancer MCF-7 cells treated with five different pharmaceuticals or toxicants [paclitaxel (PTX), gossypol (GOS), methyl jasmonate (JAS), L-nicotine (NIC) and melamine (mela)] and analyzed the stability of the selected reference genes by four different methods: geNorm, NormFinder, BestKeeper and the comparative ΔCt method. According to our analysis, a protein-coding gene, hTBCA and four non-coding genes, hRNU44, hRNU48, hU6 and hRNU47, appear to be the most reliable reference genes for the five chemical treatments. Similar results were also obtained in Dose- and time-response assay with gossypol treatment.

A-2006

SAS-6 Tetramers Self-assemble in the Formation of the Centriole Central Tubule in Drosophila. GEULAH BEN-DAVID[1], Jayachandran Gopalakrishnan[2], and Tomer Avidor-Reiss[2]. [1]Stern College for Women, Yeshiva University, 245 Lexington Ave., New York, NY, 10016 and [2]Department of Cell Biology, Harvard Medical School, 250 Longwood Ave., Boston, MA, 02115-5730. Email: gbendavi@yu.edu

Centrioles are cylindrical microtubule-rich organelles that display radial nine-fold symmetry. A pair of centrioles within pericentriolar material form the centrosome. Centrosomes are the major microtubule-organizing centers of eukaryotic cells. Centriole duplication is a process in which each centriole in the pair facilitates the production of a new centriole. It involves the establishment of an early "cartwheel" structure. This cartwheel is composed of a central tubule and molecular spokes that translate the nine-fold symmetry to the microtubule wall. It has been demonstrated that SAS-6 is a component of the central tubule, and mutations in SAS-6 eliminate the nine-fold symmetry of the centriole. Thus, it is hypothesized that the central tubule

may be responsible for forming the characteristic nine-fold symmetry of the mature centriole. In vitro experiments have demonstrated that SAS-6 self-assembles to form tetramers that are stable within the cytoplasm. It is possible that these represent intermediates in the assembly of the central tubule. To understand whether interactions between these SAS-6 tetramers are sufficient for the formation of the central tubule, several point mutations were generated in a highly conserved domain of the SAS-6 protein to inhibit the formation of the central tubule without disrupting its tetrameric self-assembly. In western blot analysis, third-instar larval brains were extracted and probed with anti-SAS-6 to understand the expression of both native and recombinant SAS-6 in each generated mutant. With this, it was determined that the mutant flies expressed the recombinant protein efficiently, allowing for further studies investigating the details of central tubule formation and centriole assembly in the specific mutant backgrounds.

A-2007

Studies of Organic Anion Transport Protein1a1: Preparation and Expression of p3xFLAG Construct. DINA GOLFEIZ[1], Pijun Wang[2], Jo Choi[2], Wen-Jun Wang[2], Aparna Mukhopadhyay[2], and Allan W. Wolkoff[2]. [1]Lexington Ave., No 215, Stern College for Women, Yeshiva University, New York, NY 10016 and [2]Morris Park Ave., No 1300, Department of Anatomy and Structural Biology, Division of Gastroenterology and Liver Diseases, and Liver Research Center, Albert Einstein College of Medicine, Bronx, NY 10461. Email: dgolfeiz@yu.edu

Organic anion transport protein1a1 (oatp1a1) is expressed on the basolateral plasma membrane of rat hepatocytes where it mediates uptake of amphiphilic molecules and xenobiotics from the blood. Oatp1a1 is a 12-transmembrane domain integral membrane protein that has a PDZ consensus site at its C-terminus and binds to PDZK1. The long-term goal of the present study is to determine whether oatp1a1 forms homooligomers. The short-term aim of this study was to prepare an expression plasmid encoding oatp1a1 in which a 3xFLAG sequence is expressed at its N-terminus. Oatp1a1, polymerase chain reaction (PCR) amplified using primers devised to add *NotI* and *KpnI* restriction sites, was inserted into the multicloning region of the p3xFLAG expression plasmid. DH5α E.coli were transformed with this plasmid and grown on agarose plates using ampicillin as the selection marker. Minipreps of randomly selected bacterial clones were performed in which extracted DNA was digested with enzymes *NotI* and *KpnI* and checked by DNA sequencing, as indicated. Several plasmids with the correct cDNA sequence were obtained and were used to transfect HEK293T cells. Forty-eight hours after the transfection, cell lysates were prepared and subjected to Western blotting to assay for expression of 3xFLAG-oatp1a1 protein using oatp1a1 and FLAG antibodies. P3xFLAG-oatp1a1 was prepared successfully and confirmed by DNA sequencing. Transfection of HEK293T cells with this plasmid revealed abundant expression of a protein that reacted with both FLAG and oatp1a1 antibodies on Western blot. In summary, a plasmid expressing 3xFLAG-oatp1a1 has been prepared successfully. Co-transfection of HEK293T cells with this plasmid and a plasmid encoding oatp1a1 linked to a different marker (e.g. GFP) will permit studies to determine whether immunoprecipitation of one form of oatp1a1 will contain the other, indicating that they are bound in a complex. This plasmid should thus provide an important tool in which to conduct oatp1a1 dimerization studies in the future.

A-2008

Effect of Ebselen in Modulating Genotoxicity in Cultured Human Cells from Chloroethyl Ethyl Sulfide Exposure Using Comet Assay Analysis. CLARK L. GROSS, Eric W. Nealley, Adele L. Miller, Mary T. Nipwoda, Offie E. Clark, and William J. Smith. U. S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010. Email: clark.gross@us.army.mil

Sulfur mustards are genotoxic and carcinogenic in addition to their cytotoxic and vesicating (blistering) properties. The genotoxicity of these alkylating agents can be measured by *in vitro* tests such as the single cell gel electrophoresis analysis (Comet assay) which measures the extent of DNA fragmentation. Compounds which change these DNA fragmentation patterns may be identified by this simple test since they modify the Comet moment of exposed cells. A number of compounds have been identified that exhibit anti-genotoxicity and work by a number of poorly understood pathways. One of the more promising compounds is ebselen, a seleno-methionine which is both an anti-inflammatory and an anti-oxidant. It has been shown effective as a co-treatment against nitrogen mustards. We wished to determine whether this compound would prove effective as a therapy for DNA fragmentation caused by chloroethyl ethyl sulfide (CEES) in normal human epidermal keratinocytes (NHEK) in culture. Cells were exposed to 500 μM CEES for 2 hrs to allow its complete hydrolysis and ebselen in DMSO was added at 1, 5, and 10 μM concentrations. Cells were incubated for an additional 24 h, harvested, and processed for genotoxicity analysis by the alkaline Comet assay. Ebselen did not have any effect on untreated cells since the Comet moment

was similar at all 3 concentrations tested. However, both 5 μM and 10 μM ebselen appeared to decrease DNA fragmentation in CEES-treated cells approximately 50 % while 1 μM decreased the fragmentation about 20% in preliminary experiments. Ebselen did not have any effect on viability as judged by propidium iodide uptake via flow cytometry. Ebselen may have a role as an adjuvant in approaches to therapy against sulfur mustard genotoxicity. This research was supported by the Defense Threat Reduction Agency—Joint Science and Technology Office, Medical S&T Division.

A-2009

Pomegranate and Olive Fruit Extracts, Prooxidants with Antiproliferative and Proapoptotic Activities Towards HSC-2 Carcinoma Cells. A. G. SCHUCK, S. S. Cohen, L. T. Lerman, O. Haken, and J. H. Weisburg. Department of Biology, Stern College for Women, 245 Lexington Ave, NY, NY 10016. Email: schuck@yu.edu

Thirty percent of all cancers are linked to poor dietary habits. The consumption of fruits and vegetables are potential chemopreventive lifestyle choices, in that their daily intake can potentially prevent cellular changes leading to cancer. The health benefits of fruits, including pomegranates and olives, have focused on their antioxidant properties, attributed to their high content of soluble polyphenols. Research in our laboratory, however, has indicated that polyphenol-containing extracts from these fruits also exhibit prooxidant activity, which could contribute to their anticarcinogenic activities. The antiproliferative, proapoptotic, and prooxidative effects of pomegranate extract (PE) and olive extract (OE) were studied *in vitro,* using HSC-2 carcinoma cells derived from the human oral cavity. Both PE and OE, albeit to a lesser extent than PE, demonstrated antiproliferative effects on HSC-2 cells. These results correlated with the higher number of reactive oxygen species (ROS) generated by PE than by OE, as determined by the ferrous oxidation-xylenol orange (FOX) assay. The antiproliferative effects of both extracts were linked to their induction of oxidative stress, as the cytotoxicities of the extracts were potentiated in cells pretreated with the glutathione depleter, 1-chloro-2,4-dinitrobenzene, and attenuated in cells cotreated with the ROS scavengers, catalase, pyruvate, and divalent cobalt ion. Oxidative stress is a trigger of cell death, typically by apoptosis. PE and OE induced apoptotic cell death, as demonstrated by activation of casapase-3 and cleavage of PARP. Decreases in caspase-3 activation and PARP cleavage occurred in cells cotreated with PE or OE and either cobalt or pyruvate, as compared to treatments with PE or OE alone.

A-2010

Long-term Serial Cultivation of Mouse Induced Pluripotent Stem (iPS) Cells in Serum-free and Feeder-free Defined Medium. SACHIKO YAMASAKI[1], Miho Kusuda Furue[2], J. Denry Sato[3], and Tetsuji Okamoto[1]. [1]Department of Molecular Oral Medicine and Maxillofacial Surgery, Division of Frontier Medical Sciences, Graduate School of Biomedical Sciences, Hiroshima University, 2-3, Kasumi-1, Minami-ku, Hiroshima 734-8553, JAPAN; [2]Laboratory of Cell Cultures, Department of Disease Bioresources, National Institute of Biomedical Innovation, Osaka, JAPAN; and [3]Manzanar Project. Email: sayamasaki@hiroshima-u.ac.jp

Mouse Embryonic Stem (mES) cells and mouse induced Pluripotent Stem (miPS) cells are commonly maintained on inactivated mouse embryonic fibroblast feeder cells in medium supplemented with FBS or proprietary replacements. Use of culture media containing undefined or unknown components has limited the development of applications for pluripotent cells because of the relative lack of knowledge regarding cell responses to differentiating growth factors. Therefore we developed a serum-free medium, designated ESF7, in which mouse ES cells can be maintained in undifferentiated state without feeder cells. Using this culture condition, the miPS cells are able to be maintained in ESF7 medium for more than 1 yr with an undifferentiated phenotype by virtue of the expression of the transcription factor Oct-3/4, Nanog, Sox2, Esg1, and alkaline phosphatase, and the cells exhibited largely normal karyotypes. Furthermore, we found that fibroblast growth factor (FGF)-2 with heparin induced miPS cells to differentiate into neuronal- and glial-like cells both in an adherent monolayer culture and in suspension embryoid body culture. miPS cells maintained both in ESF7 and in conventional serum-supplemented medium with feeder cells transplanted subcutaneously into the back skin of SCID mice, both produced tumors with tissues of all three germ layers. As this simple serum-free adherent monoculture system supports the long term propagation of pluripotent iPS cells in vitro, it will allow us to elucidate the cell responses to growth factors under defined conditions, and could provide useful information to develop the feeder-free and serum-free medium of human iPS cells for clinical use.

A-2011

Selection of Reliable Reference Genes for Quantitative PCR Analysis *for Nanotixicity Study* in *C.elegans*. Y. Q. ZHANG, B. H. Zhang, and X. P. Pan. Department of Biology, East Carolina University, Greenville, NC 27858. Email: ZHANGY10@students.ecu.edu

With rapid development and application of a wide range of manufactured metal oxide nanoparticles(NPs), limited infor-

mation is known about the risk of using NPs. In order to investigate the potential adverse effects of NPs, we use *C. elegans* as a simple and well biologically defined animal mode to analysis gene expression differences. Real-time PCR is one of the most powerful methods for gene expression studies. The most important factor that may affect this highly sensitive tool is to choose appropriate normalization. In this study, we selected 13 reference gene candidates (act-1, cdc-42, pmp-3, eif-3.C, actin, act-2, csq-1 Y45F10D.4, tba-1, mdh-1, ama-1, F35G12.2) and tested their expression in four different doses with nano-copper oxide (0, 0.001, 0.01 and 0.05 mg/ml) using quantitive PCR. Four different methods, geNorm, NormFinder, BestKeeper and the comparative ΔCt method, were employed to evaluate the 13 candidates expression stability. As a result, tba-1 and Y45F10D.4 showed most reliable in this study, which may be used in the future for nanotixicty study as well other toxicity studies.

A-2012

Functional Role of the Notch Signaling Target Gene HRT2 in Vascular Smooth Muscle. CATHERINE A. BLEBEA, Suhanti Banerjee, and Aaron Proweller. Division of Cardiology, CWRU School of Medicine, Cleveland, OH. Email: catblebea@gmail.com

Background: The Notch signaling pathway is important for the control of cell differentiation and proliferation, particularly in arterial vascular smooth muscle cells (VSMCs). We hypothesized that overproduction of the Notch target gene, Hairy-Related Transcription factor two (HRT2), may result in a proliferative response improve cell growth and development. Materials and Methods: VSMCs were isolated from freshly harvested aortas from control wild-type (WT; n=3) and transgenic ($HRT2^{Tg}$ n=2) mice. Each aorta was cut into 500 micron slices (n=35 total), placed into culture media for VSMC growth and proliferation. Cells were passaged once and seeded onto 24-well plates. Cells were then placed on cover slips for 48 h in low (DMEM/F12/1% FBS) serum conditions for growth challenging conditions and then fixed with 4% paraformaldehyde. Primary immunohistochemical staining was done with anti-phospho-histone H3 (P-H3) antibody to identify nuclear mitoses, a marker for cell proliferation activity, and secondary fluorescent antibody for visualization. Following 48 h of antibody incubation, the cells were counted under 1000x magnification. Results: The transgenic $HRT2^{Tg}$ cell population was found to grow significantly faster as measured by confluency rates, (Figure 1) reaching 67% at six days as compared to 25% in the controls, and 85% vs 15% at nine days, respectively [$p<0.001$, ANOVA]. Control wild-type failed to survive growth-challenging low-serum conditions (Figure 2), with only a 25% survival rate compared to 100% of the $HRT2^{Tg}$ cells [$p<0.05$, Fischer Exact test]. Conclusions: The transgenic mice, with an overproduction of the Notch target gene, HRT2, were significantly hardier, with faster cell proliferation, than the control group. This suggests a potential therapeutic role for stimulation of the Notch pathways to increase vascular embryologic development in deficient states, or reduce it in hyperproliferative states, such as in peripheral arterial disease or following cardiac angioplasty or stenting.