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CO-EXPRESSION OF C-*MET* AND HGF/SF IN MOUSE MUSCLE CELLS AND THE EFFECT OF THE CONSTITUTIVE *MET* KINASE ACTIVATION ON MYOGENIC DIFFERENTIATION (S. Anastasi, S. Giordano°, O. Sthandier, R. Maione, P. Comoglio° and P. Amati) *Dipartimento di Biopatologia Umana,, Università di Roma La Sapienza, 00161 Roma and ° Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Torino, 10126 Torino, Italy*

We will report on the expression of both *c-met* and HGF/SF in *in vitro* actively growing C2 mouse myoblast cells. The high degree of tyrosine phosphorylation exhibited by *c-met* receptor suggests that HGF/SF may have an influence on C2 myoblasts through an autocrine loop. Moreover, we have found that the transcription of *c-met* and HGF/SF genes is repressed when myoblasts stop proliferating and differentiate upon serum deprivation.

To investigate the role of HGF/SF in muscle differentiation, we studied the effects of the ectopic expression of human *c-met* (h-*c-met*) and human HGF/SF (h-HGF/SF) in stable transfected C2 cells. The constitutive expression of single human isoforms does not alter the growth and differentiation properties of the myoblast cells. Stable double expressing h-*c-met* and h-HGF/SF clones are phenotypically identical to those expressing *Tpr-met*, the truncated activated cytoplasmic form of h-*c-met*. In both cases cells alter their morphology when grown at low density, evidentiating the morphogenic and motogenic activity. However, upon growth to confluence cells are contact inhibited and differentiate inversely to the degree of expression of the heterologous genes. Our data demonstrate that the establishment of a species-specific autocrine loop for h-HGF/SF or the expression of a costitutive activated form of the receptor, released from differentiation-dependent expression control, confers a modified phenotype to myoblast cells and inhibits muscle differentiation.

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ERYTHROID DIFFERENTIATION AND DENUCLEATION FACTORS FROM FETAL RAT LIVER: MONOCLONAL ANTIBODIES PREPARATION FOR CLONE SCREENING FROM HUMAN BONE MARROW cDNA LIBRARY. ((S.S.W.Chan, S.F.Zhang, M.F.Li, J.Zuo, R.M.W.Chau)) Department of Anatomy, The University of Hong Kong, Hong Kong.

The methods of "protein band-fishing by cells", Wright's stain differential cell count, and MTTcolorimetric assay were used to detect, identify, and follow the biological activities of the erythroid differentiation and denucleation factors (EDDFs) from the extract of fetal rat liver. Native proteins from the extract were electrophoretically separated into protein bands in the Phastgel. Onto the surface of such Phastgel $5x10^6$ murine erythroid leukemia (MEL) cells were seeded and cultured for 6 days allowing for differentiation and denucleation. The cell culture was fixed together with the Phastgel and studied under an inverted microscope for degree of differentiation and denucleation. The result suggested that at the regions of molecular weight 17 and 94 kD on the Phastgel there were more denucleated or mature red blood cells present. In order to verify the presence of EDDF activities in these regions, such Phastgel were cut into 50 stripes from top to bottom and each stripe was used to co-culture with MEL cells. Results revealed that 60-70% of the MEL cells in the cultures containing the protein band of 17 or 94 kD were differentiated and denucleated into reticulocytes and mature RBCs as compared to ~20% in cultures with control gel stripes. In order to confirm the specific EDDF activity in the 17 and 94 kD gels, they were cut out and used as antigen to immunize BALB/c mice to prepare for EDDF monoclonal antibodies. Two clones each of hybridomas were selected by the capability of their Mabs in blocking the differentiation and denucleation activities of EDDFs in the extract or gel stripes of 17 or 94 kD, in the MEL cell culture. About 70-74% of MEL cells remained undifferentiated in the cultures with the EDDF and the specific Mab as compared to ~20% in the control cultures with no Mab. The results suggested that we have obtained specific Mabs capable of blocking the differentiation and denucleation activities of the 17 or 94 kD EDDFs. Two clones each from the human bone marrow cDNA library were selected by these Mabs for EDDF gene structure and function studies. (Supported by RGC, GF, and CRCG grants).

Senescence (3101-3104)

3101

LONG-LIVING LENS PROTEINS AND NEW CONCEPT OF AGING ((A.V.Khalyavkin and A.V.Blokhin)) Division of Kinetics of Chemical & Biological Processes, Institute of Chemical Physics of RAS, Moscow 117977, Russia.

An analysis of the facts taken as a whole shows that they are compatible with the concept that living conditions exist which are conducive to a total non-senescence of the complex organism. In accordance with this view the aging of the living organisms is the consequence of the influence of external factors that induce organisms to function in non-optimal physiological regimen. This concept cover any species with a life history that incorporates repeated reproduction and, in particular, is supported by the limitless proliferative potential of somatic stem cells and by the fact that mortality pattern for the real organisms is exactly the same as the mortality pattern for hypothetical populations of potentially non-senescent organisms, which do, however, experience senescence as a result of functioning in living conditions that to varying degrees prevent the total self-maintenance of the organisms. The weak spot of this concept is the lack of protein synthesis in the differentiated lens fiber cells. Since lenticular cells are never shed, the lens nucleus or core contains cells as old as the organism itself. In contrast to the rapid rate of protein turnover in many organs, the proteins in lens exist in situ for decades. In this connection they must be undergone inevitable posttranslational modification and denaturation. However it does not happen in norm, because the lens proteins - crystallins are not passive structural entities, but are functional components of long-living self-maintaining multimolecular system. Some kinds of crystallins are examples of the recruitment of enzymes, chaperones, heat shock proteins and other functional polypeptides to a new structural role. The functional activity of the crystallins in optimal humoral conditions may protect them against denaturation and photochemical damage. That is to say, the features of crystallins are compatible with the above-mentioned concept of aging.

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REGULATION OF E2F RELATED GENES DURING CELLULAR SENESCENCE ((G. P. Dimri, M. Acosta and J. Campisi)) Department of Cancer Biology, Berkeley National Laboratory, University of California, Berkeley CA 94720

A limited cell division potential is a hallmark of most normal eukaryotic cells. This property of normal cells is known as replicative senescence. The exact causes of replicative senescence are not known. We present data that suggest that the failure of senescent cells to express certain members of the E2F gene family may play a role in senescence. First, we observed the downregulation of E2F binding activity in senescent cell nuclear extracts. This downregulation of E2F binding activity probably is due to failure of senescent cells to express E2F1, a pRB binding protein and E2F5 , a P130 and P107 binding component of E2F. E2F4 also fails to show a growth specific upregulation in senescent cells. We are further determining the role of E2F genes by constitutive overexpression of the E2F gene family members using retroviral vectors in near senescent cells. Our working hypothesis is that the E2F genes may extend the *in-vitro* life span of cells, which may be a critical step for immortalization. Our preliminary results indicate that the overexpression of members of E2F gene family particularly E2F1 and E2F5 induces deregulated growth which ultimately leads to cell death by some unknown mechanisms. We also studied the effect of overexpression of E2F genes in immortal mouse 3T3 cells. E2F1 and E2F5 induces cell death in 3T3 cells. However, in 3T3 cells death appears to be due to induction of an apoptotic pathway. This result indicates that there are important differences between normal and immortal cells with respect to overexpression of growth regulatory genes and induction of cell death pathways. Finally these results support the idea that the cell needs a delicate balance of positive and negative growth regulators. We are exploring the possibility that certain viral oncogenes such as E6 and E7 or antideath cellular genes together with E2F, may provide such a balance.

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THE VIRTUAL CELL LABORATORY PREDICTS THE MOLECULAR MECHANISM OF CELLULAR SENESCENCE — A COMPUTER-AIDED APPROACH TO BIOLOGY OF AGING ((H. Kitano, and S. Imai)) Sony Computer Science Laboratory, 3-14-13 Higashi-Gotanda, Shinagawa, Tokyo, 141 Japan, and Department of Microbiology, School of Medicine, Keio University, 35 Shinanomachi, Tokyo, 160 Japan

We propose a new hypothesis on cellular senescence and show its validity by using the Virtual Cell Laboratory (VCL), a computer simulation system for the molecular dynamics of cells. Our hypothesis is that cellular senescence is caused by a combination of two distinct, but time-aligned mechanism – stochastic growth arrest (SGA) and catastrophic senescence (CS). Both mechanisms are variations of a more fundamental transcriptional regulatory scheme — the DNA Replication Dependent Transcriptional Control (RDTC). In our model, sequential release from the transcriptional repressive status is driven by RDTC, which results in the induction of a certain kind of growth inhibitory effector genes. SGA is caused by the local cell-to-cell interaction mediated by the membrane-associated type of growth inhibitory effector gene product. CS is cell autonomous growth arrest driven by the gene product(s) regulated directly by RDTC. We have developed VCL, which implement abstract form of transcriptional regulatory structures in each of 10^5 virtual cells. VCL actually reproduces cellular internal conditions, such as numbers of repressors or nucleosomal components, how they repress the growth inhibitory effector genes, and how they are distributed to nascent daughter strands at each replication cycle. We found that our hypothesis can explain and reproduce three distinct growth kinetics and gene expression patterns; normal, SV-40 T antigen-transformed, and Werner's syndrome (WS) fibroblasts. Several predictions were made by VCL: (1) cell-to-cell interaction is necessary to reproduce gradual increase in the fraction of non-dividing cells in cell population, (2) SV-40 T antigen can postpone the timing of CS-associated gene expression, resulting in prolonged life span of cell population without changes of individual cell life span, (3) the mutation of WS gene may possibly increase the probability that transcriptional components being lost.

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OCT1-MEDIATED TRANSCRIPTIONAL REPRESSIVE MACHINERY CONTROLS CELLULAR SENESCENCE- AND IMMORTALIZATION-ASSOCIATED GENE TRANSCRIPTION ((S. Imai, T. Fujino, S. Nishibayashi, K. Takao, M. Hasegawa, and T. Takano)) Department of Microbiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo-160, Japan

To study the mechanism of cellular senescence and immortalization, it is important to focus on the cellular senescence- and immortalization-associated transcriptional regulatory pathways. In our experimental system of SV40 T antigen-transformed human diploid fibroblasts, we found that loss of Oct1-mediated transcriptional repression seems to trigger the cellular senescence-associated collagenase gene expression. We also found that this Oct1-mediated transcriptional repression is resumed in the process of immortalization. The cellular senescence- and immortalization-coupled regulation of the collagenase gene suggests that the transcriptional regulators of this gene may play important roles in the mechanism of both processes. Oct1 recognizes the sequence AAATAATT in the immortalization-susceptible *cis*-element (ISE) 2 in the collagenase upstream region. Transcriptional activity of the collagenase upstream region is repressed by overexpression of oct1 cDNA in a dose-dependent manner. In addition, we identified another ISE2-binding factor SSRP1, a member of HMG-box protein family, which was demonstrated to mediate transcriptional repression of the collagenase gene in cellular senescence- and immortalization-associated manner. Oct1 may possibly interact with this HMG-box factor. In cellular aging of yeast, loss of transcriptional silencing was demonstrated to cause an aging-specific phenotype of cells. Therefore, we suggest that Oct1-mediated transcriptional repressive machinery is substantially involved in the molecular mechanism of cellular senescence and immortalization.