49 Molecular Cloning of a Novel Gene in Endothelial Cells. R. PRAMANIK, M.J. FEKETE, M.W. LINGEN* (Loyola Univ. Medical Center, Maywood, IL).

Retinoids, such as all-trans-retinoic acid (ATRA), have profound effects on the physiology of all cell types including endothelial cells. We are interested in identifying and studying genes that are differentially expressed in endothelial cells during the induction and inhibition of angiogenesis. In order to identify candidate genes that may be involved in this process, mRNAs from early passage human dermal microvascular endothelial cells (HMVEC), cultured with either nothing (control), 1 uM ATRA, conditioned media (CM) from a human oral squamous cell carcinoma cell line (SCC-9), or both ATRA and CM were amplified by reverse transcription-polymerase chain reaction (PCR) and compared via differential display. PCR products whose expression was modulated as a result of the various treatments were used as probes to screen a passage 2 HMVEC cDNA library. One of the full-length clones obtained was a novel gene that was designated RARG-1. Two RARG-1 transcripts of 1.4 and 0.9 kilobase pairs were observed on Northern blot analysis. In addition to HMVECs, RARG-1 mRNA is expressed variably but ubiquitously in 19 different human tissues. The open reading frame encodes for 289 amino acids corresponding to a putative protein of 32.8 kDa. The predicted protein is basic (pI=9.92). The most abundant amino acids are lysine (14.3%), glutamine (10.1%), alanine (9.1%), and arginine (8.7%). These results suggest that RARG-1 is a novel gene that is in all human tissues tested. Supported by USPHS grant DE12322

50 Initiation of Intraepithelial Neoplasia Requires Escape From Microenvironmental Control. M.VACCARIELLO, T. KOLODKA, Y. WANG, N.E FUSENIG, J. GARLICK*, (SUNY Stony Brook and German Cancer Research Center, Heidelberg).

We previously reported that interactions with normal human keratinocytes (NHK) can control neoplastic progression of early-stage transformed keratinocytes in stratified epithelium (*Can.Res.*58:2200-2209,1998). The purpose of this study was to determine if late-stage transformed keratinocytes could also be controlled by the microenvironment and to begin to elucidate the mechanism for growth control. To accomplish this, we mixed genetically-marked (β-gal) low-grade (II-4) or high-grade (SCC-13) malignant keratinocytes with NHK at varying ratios in organotypic cultures and in nude mice transplants. Tumor cell behavior was monitored by immunofluorescence staining for β-gal to measure expansion and proliferation (BrdU) as well as expression of keratin 1 (K1), Desmoglein-3 (DSG3) and Connexin 43 (CX43). In addition, studies were conducted to determine whether diffusible factors or direct cell contact were associated with tumor control. We found that II-4 cells were growth-suppressed at ratios at which SCC13 continued to undergo expansion *in vitro* and invasion after grafting, suggesting that the stage of transformation, and not the initial number of tumor cells present that determines the malignant potential of a tumor cell population. Clusters of expanding SCC13 cells continued to proliferate in the absence of adhesion to basement membrane and did not express K1, DSG3 or CX43. NHK-conditioned medium did not suppress tumor cell expansion while direct contact between NHK and tumor cells was needed for suppression. We conclude that initiation of cancer progression is due to the greater degree of autonomous growth acquired by late stage tumor cells (SCC13) resulting in their escape from cell contact-mediated microenvironmental control. (Supported by grants from NIDR (DE-11250-03) and Smokeless Tobacco Research Council (0707-02).

51 Basic Fibroblast Growth Factor (bFGF) Induced Angiogenesis Facilitates Tumorigenecity. S.R.MALLERY*, P. PEI, G. ZHU, G.M.NESS, S.P.SCHWENDEMAN (Ohio State University, Columbus, Ohio)

The failure of anti-HIV combination therapy is now being accompanied by a resurgence of AIDS-related Kaposi's sarcoma (KS). Currently there are no animal models for KS, and mucocutaneous KS cell isolates have proven nontumorigenic in animal hosts. However, the successful culture of KS cells in animals would provide an *in vivo* environment for evaluating novel treatment modalities. The purpose of this study was to determine whether bFGF induced angiogenesis facilitates the growth of KS cell strains in a murine model. For these preliminary studies 4 BALB/c male mice were used. All animals received 200 µl Matrigel® at the subcutaneous lower back implant sites. Every mouse had both a control and an experimental side, comprised of blank polylactide-co-glycolide (PLGA) millicylinders or PLGA bFGF releasing millicylinders (determined to release in a controlled fashion 1.4 µg biologically active bFGF over 7 days), respectively. At the experimental side, mice 1 & 2 received 200 µl Matrigel®without KS cells, while mice 3 & 4 received 1 x 10^6 KS cells (early passage derived from a biopsy confirmed AIDS-KS lesion) in 200 µl Matrigel®. The animals were sacrificed 7 days after millicylinder placement, and tissues processed for analyses. Control tissues showed mild inflammation, only evident microscopically. In contrast, neovascularization was noted both grossly and microscopically at all of the bFGF millicylinder sites. Further, mice 3 & 4 developed subcutaneous nodules which microscopically recapitulated KS lesions i.e., a richly vascularized stroma with mitotically active spindle cells. In situ hybridization (InnoGenex ISH Kit, specific for human DNA Alu elements) confirmed the human origin of the proliferating spindle cells. Longer duration studies are ongoing to compare the tumorigenic facilitation of controlled release vs. bolus bFGF delivery. This study reports the ability to culture "native" KS cells (not derived from pleural effusions stringently selected for the most aggressive phenotypes) in a murine model. We speculate that the bFGF induced angiogenesis significantly enabled KS cell growth. Finally, this technique, which permits *in vivo* culture of early passage, mucocutaneous derived KS strains, will permit therapeutic evaluations on cells that more closely resemble the cellular populations comprising the actual KS lesion. This study was supported in part by DE RO-1 12183.

52 **VEGF EXPRESSION BY KELOID FIBROBLASTS.** D.V. MESSADI*, A. LE, P. KELLY and C.N. BERTOLAMI. (University of California, Los Angeles and San Francisco Schools of Dentistry, Charles Drew University, California, USA)

Keloids can be classified clinically into two lesional sites, an erythematous outer border (inflammatory) and an inner non erythematous or stable center (classical keloid). Histologically, the stable center of the keloid is highly collagenous, hypovascular, and relatively hypocellular. In contrast, the inflammatory border contains large populations of lymphocytes and fibroblasts interspersed within a dense capillary network. Vascular endothelial growth factor (VEGF), a selective endothelial cell-specific cytokine, exhibits both *in vitro* and *in vivo* angiogenic capability as well as increasing vascular permeability, which are important features of the wound healing process. In this study we examined the effects of TNF-α and hypoxia, both known to exist in the early wound milieu, in the regulation of VEGF expression by keloid fibroblasts. Three different cell strains, normal skin fibroblast (NSK), keloid fibroblast derived from the inflammatory border and stable center were examined. Cells were exposed to TNF-α (50 ng/mL), hypoxic condition (2% O_2) and normoxic condition (20% O_2) for 24 hours. Expression of VEGF and VEGF receptors was examined using ribonuclease protection assay (RPA) with specific riboprobes (hAngio-1 Template set, Pharmingen, San Diego, CA). Our results indicate: 1) both NSk and keloid fibroblasts express VEGF ligand but not VEGF receptors; 2) Both TNF-α and hypoxia upregulated the expression of VEGF in both NSK and keloid fibroblasts; 3) keloid fibroblasts derived from the inflammatory border consistently express a higher level of VEGF than fibroblasts from the stable center. These findings suggest that the increased VEGF production by keloid fibroblasts present in the inflammatory border may lead to the proliferation of endothelial cells that occlude the microvessels in the center of keloid and contribute to the biology of keloid formation. This work was supported by NIH/NIDR grant DE 01598.

53 Patients' Pre- and Post-Treatment Preferences for Mandibular Prostheses. M.A. AWAD, J.P.LUND, J.S. FEINE* (Faculty of Dentistry, McGill University, Montreal, Canada).

Several researchers have suggested that treatment preference should be incorporated into study designs and considered in the analyses. This paper is a report on a comparison of pre- and post-treatment preferences for two types of dental prostheses. Edentulous subjects (n=102) who participated in a randomised clinical trial comparing mandibular conventional dentures (CDs) and overdentures supported by two implants (IOs) were asked, prior to randomisation, which treatment they would prefer if given a choice. Sixty-one% preferred IOs, 15% preferred CDs and 24% were neutral. The groups were stratified so that half of each of the preference groups received each treatment. Two months after subjects had received their prostheses, they were then asked to indicate which treatment they now preferred. Overall, most of those who wanted and received IOs maintained their original preference (88%), while 66% of those who had preferred CDs changed their preference after having received them (p=0.04). Of those who were neutral prior to treatment, 92% who received the IOs now preferred implant therapy, while only 44% of the neutral group expressed a preference for CDs after receiving them (p=0.002). We previously reported that those who preferred IOs expected these to outperform CDs, whereas there were no significant differences in treatment expectation for the CD and neutral groups. The results of this analysis suggest that IOs met or exceeded the expectations of subjects who preferred this treatment, while CDs did not meet the expectations of those who wished for it or of those who had no preference. Supported by the Canadian MRC, Health & Welfare Canada (NHRDP) and Nobel Biocare Canada Inc.

54 A One-Year Follow-up Analysis of the Efficacy of Implant Overdentures. M.A. AWAD*, J.P. LUND, E. DUFRESNE, J.S. FEINE (Faculty of Dentistry, McGill University, Montreal, Canada).

In a randomised controlled clinical trial to compare the performance of mandibular conventional dentures (CDs) with overdentures on two osseointegrated implants connected by a bar (IOs), we reported that subjects in the IO group (n=54) were significantly more satisfied with their prostheses than those who received CDs (n=48; p<0.05), and that the IO group had significantly higher ratings of health-related quality of life using the Oral Health Impact Profile (OHIP) questionnaire 2 months after delivery of the prostheses. Here, we report on data gathered using the same instruments one year later. In general, the scores given 2 months after delivery of the prostheses were unchanged at one year for almost all variables. Subjects who received IOs (n=36) were more satisfied with their prostheses than subjects who received CDs (n=22; ps<0.05). Significant differences between the two groups were also observed for ratings of comfort, stability and ease of chewing (ps<0.05). Furthermore, patients in the IO group reported lower overall OHIP scores than those in the CD group (p<0.05). In particular, differences in favour of IO therapy were observed between the two groups on 5 specific domains of the OHIP: functional limitation, pain, psychological discomfort physical disability and psychological disability (ps<0.05; t-test). There was a tendency for patients in the CD group to seek more follow-up treatment than patients in the IO group (p=0.10, t-test). The results of this follow-up analysis suggest that, after one year, mandibular 2-implant overdentures still deliver significantly greater benefits to patients than conventional dentures. Supported by the Canadian MRC, Health and Welfare Canada (NHRDP) and Nobel Biocare Canada Inc.

55 Subjective Importance of Oral Health Impacts Among Patients Receiving Dentures. G.D. SLADE* (Dept of Dental Ecology, Univ North Carolina, Chapel Hill, USA).

While reported frequency of dysfunction, discomfort and disability can quantify the social impact of oral conditions, perceived importance of those impacts may constitute an additional dimension of oral health related quality of life (OHRQoL). This study aimed to determine change in frequency and importance of oral health impacts among edentulous patients receiving replacement complete dentures in a private dental practice. Prior to treatment, 95 patients (aged 40-86 yrs, median=65 yrs) completed a questionnaire comprising: the 14-item Oral Health Impact Profile, which asks about frequency of dysfunction, discomfort and disability due to oral problems within the preceding month; corresponding questions about the importance of those impacts for overall enjoyment of life; and questions about satisfaction with dentures. Responses were made on Likert scales, and mean scores for frequency, importance, and satisfaction (respectively) were computed. One year after treatment, 34 patients completed identical questionnaires. At baseline, patients reported a mean frequency of 4.8 (sd=4.4) impacts while mean importance on a 0-3 scale was 1.5 (sd=1.2). Frequency and importance ratings were moderately correlated (Pearson's ρ=0.33, P<0.01). Although frequency of impacts was greater among patients who were dissatisfied with their old dentures (mean=5.8, sd=3.7) compared with patients who were not (mean=3.7, sd=3.9, t-test P=0.03), importance was not associated with dissatisfaction (P=0.65). After receiving new dentures, frequency of impacts reduced (mean=2.8, sd=3.6, paired t-test P=0.05) but importance was similar (mean=1.2, sd=1.2, P=0.20) compared with baseline. However, both frequency and importance were significantly associated with dissatisfaction with new dentures (P<0.05). In these patients, frequency and importance of oral health impacts were only moderately correlated and displayed different patterns of change after treatment, suggesting they represent separate dimensions of OHRQoL. Supported by NIDCR R29-DE12366.

56 Translation and validation of the Chinese (HK) version of OHIP. M.C.M. Wong*, E.C.M. Lo, A.S. McMillan (Faculty of Dentistry, The University of Hong Kong)

The Oral Health Impact Profile (OHIP) is a widely used instrument for measuring oral health related quality of life. It is a 49-item questionnaire with seven domains. Subscale and summary scores can be calculated, with a higher score indicating a larger impact of oral health on quality of life. In order to facilitate the translation of OHIP into colloquial Chinese, 3 focus group discussions were conducted to collect information on how the elderly expressed their concerns about oral health issues. OHIP was then translated into colloquial Chinese and back translated into English. The translated Chinese version of OHIP was pilot tested in elderly patients attending a dental hospital in Hong Kong. Problems with the translated version were noted and modifications were made. For validation, 604 elderly aged 60-80 years were interviewed by two trained interviewers in elderly homes and social centers. Internal consistency of the seven OHIP subscales, as measured by Cronbach's alpha, ranged from 0.69 to 0.85. Elderly who perceived themselves as having better oral health had lower mean subscale scores than those who did not ($p < 0.001$ in all subscales). Elderly who perceived themselves as having dental treatment needs had higher mean subscale scores than those who did not ($p<0.001$ in all subscales). This study showed that the reliability and validity of the translated OHIP was good and it may be used as an effective instrument for measuring oral health related quality of life in Chinese. (Supported by CRCG-HKU & RGC, HKSAR)