2252nd Conference

Bio America & CRISPR-2018

24TH Biotechnology Congress: Research & Innovations

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Annual Congress on

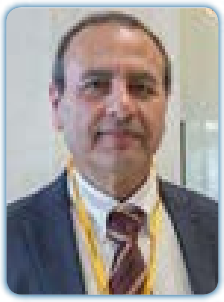
CRISPR Cas9 Technology and Genetic Engineering

October 24-25, 2018 | Boston, USA

Keynote Forum

Day 1

Fuad Fares, J Biotechnol Biomater 2018, Volume 8
DOI: 10.4172/2155-952X-C4-096



Fuad Fares

University of Haifa, Israel

Recombinant proteins: From bench to clinics

Recombinant proteins from the use of DNA technology are found in essentially every western pharmacy, medical testing laboratory, and biological research laboratory. One major issue regarding the clinical use of many peptides is their short half-life due to the rapid clearance from the circulation. To overcome this problem, we succeeded to ligate the signal sequence of O-linked oligosaccharides to the coding sequence of the hormones. The cassette gene that has been used contains the sequence of the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin β (hCGβ) subunit. The CTP contains 28 amino acids with four O-linked oligosaccharide recognition sites. It was postulated that O-linked oligosaccharides add flexibility, hydrophilicity, and stability to the protein. On the other hand, it was suggested that the four O-linked oligosaccharides play an important role in preventing plasma clearance and thus increasing the half-life of the protein in circulation. Using this strategy we succeeded to ligate the CTP to the coding sequence of follitropin (FSH), thyrotropin (TSH), erythropoietin (EPO) growth hormone (GH) and thus to increase the longevity and bioactivity of these proteins in-vivo. Interestingly, the new analogs of FSH and GH were found not immunogenic in human and it is already passed successfully clinical trials phase III and phase II respectively. Moreover, FSH long-acting was approved by the European Commission (EC) for treatment of fertility. In addition, our results indicated that long-acting GH is not toxic in monkeys and the results from the clinical trials phase I and phase II seem to be promising. Designing long-acting peptides will diminish the cost of these drugs and perhaps reduce the number of injections in the clinical protocols.

Biography

Fuad Fares have completed his MSc and DSc studies at the Faculty of Medicine, Technion-Israel Institute of Technology, and postdoctoral studies at the Department of Molecular Biology and Pharmacology, School of Medicine, Washington University, St. Louis Missouri, USA. He developed the Molecular Genetic Laboratory at Carmel Medical Center, Haifa, Israel. Now he is the head of Molecular Genetic Laboratory at the Department of Human Biology, University of Haifa, Israel. He published more than 100 manuscripts in reputed journals and 12 patents. He served as a member of the Israel Council for Higher Education last 15 years. Moreover, he is the founder and the inventor of PROLOR Biotech Company for "designing long-acting recombinant proteins". PROLOR had an exit to OPKO Health Company in the USA. He developed long-acting FSH in the USA and this hormone (ELONVA) is marketed since 2010 by Merck Germany.

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Sonali Bhattacharjee

Cold Spring Harbor Laboratory, USA

Investigating the nexus between DNA repair pathways and genomic instability in cancer

DNA double-strand breaks are one of the most lethal lesions to a cell that can be repaired by one of the two cellular pathways; non-homologous end joining or homologous recombination. Homologous recombination genes are particularly attractive targets for precision cancer therapy because these genes have altered expression patterns in cancer cells when compared with normal cells and these genetic abnormalities can be targeted for selectively killing cancer cells while leaving normal cells unscathed. Synthetic lethality is thought to be the new frontier of cancer therapeutics because it overcomes the limitation of chemotherapy, which is unable to discriminate between cancer cells and normal cells. Two genes are synthetically lethal when simultaneous disruptions of both genes give rise to a lethal phenotype, while the disruption of either gene alone is viable. Many homologous recombination genes have synthetic lethal relationships with oncogenes and tumor suppressor genes, which can be targeted for the development of cancer therapy- an approach referred to as combination therapy. In my presentation, I will summarize recent progress in understanding both the functioning and the regulation of the DNA repair machinery and elaborate on the clinical applications of these proteins in cancer therapy.

Biography

Sonali Bhattacharjee did her BSc in Biotechnology from Bangalore University in 2006 and MSc in Applied Genetics from Bangalore University in 2008. She then moved to England to pursue her DPhil (Phd) in Biochemistry from Oxford University where she studied the role of Fml1 and its partner proteins MHF1 and MHF2 in promoting genome stability. She was awarded her DPhil in 2012. During her time at Oxford, she was also a tutor at Greene's College, Oxford. In 2013, she moved to Cold Spring Harbor Laboratory, New York. At CSHL, her work has focused on understanding the epigenetic regulation of DNA repair. She is also an academic tutor at the Watson School of Biological studies, the school for graduate studies at CSHL

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Shaon Ray Chaudhuri

Tripura University, India

Dairy Effluent conversion into biofertilizer using tailor made microbial consortium

Statement of the Problem: Dairy industry generates $3m^3$ of effluent per m^3 of processed milk which comes mostly from the cleaning process that uses fresh water. The effluent is nutrient rich and can cause an environmental problem unless properly treated. The conventional treatment is tedious, energy intense, cost ineffective. Adopting this technology is a burden for the larger establishments and crippling for the small-scale installations. Hence technologies are sought to make the process of effluent treatment eco-friendly and economically viable.

Methodology & Theoretical Orientation: Tailor-made consortium was developed for conversion of the nitrogenous waste in dairy effluent into ammonia. The process was carried out using biofilm bioreactors in order to ensure one-time bacterial charging with continued performance. The process was scaled to industrial scale (more than 5000 liters). The treated effluent was used for field trial and pot trial for the cultivation of economic crops as per standard procedure.

Findings: Tailor-made microbial consortium produced ammonia from dairy effluent at a rate of $1.66x10^{-4}$ mol s^{-1} within 1 hour of incubation in a biofilm bioreactor at 37°C with highest production at 16th hours of incubation (56.81mg/100ml) demonstrating 95.7% ammonia production with 72.3% nitrate and a concomitant 33.2% phosphate reduction from an initial load of 32-270ppm nitrate and 15-40ppm phosphate respectively with 82.55% BOD reduction in 16th hour, as compared with 66.6% in 48 hours through constructed wetlands. The treated effluent increased biomass in case of mung bean (Mb) and Sorghum Sudan grass; decreased root nodulation while enhanced seed yield with improved protein and carbohydrate content in Mb while providing protection from aphid infestation. This treated effluent significantly enhance the basal diameter and fiber yield in case of Ramie, a plant of immense economic value. It could also enhance production in the case of potato, hence functioning as a biofertilizer. This approach enables the conversion of effluent into a by-product of immense economic value hence making the process of dairy effluent treatment self-sustainable. The process was scalable from 1liter to more than 5000 liters for treating actual dairy effluent with associated field trial.

Biography

Shaon Ray Chaudhuri from the Department of Microbiology has been working independently in the area of Microbial Technology since 2003. Her group has been working on development waste water specific tailor made microbial consortium for treatment with minimum dead mass generation. Eight scholars have graduated; two are in the process of being awarded their doctoral degree, while 6 scholars and 9 master's students are working in the group to develop new solutions for waste management with environmental sustenance. She has 5 technologies transferred, 4 awarded international patents while 10 filed patents.

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Francesca Storici

Georgia Institute of Technology, Atlanta, USA

DNA break repair and modification guided by transcript RNA

Does genetic information flow from RNA to DNA in a more general fashion than anticipated? Is the central dogma of molecular biology often reversed to let RNA repair DNA damage or even recode genes on chromosomes? We recently discovered that RNA serves as a template to repair DNA double-strand breaks (DSBs), either indirectly, in the form of complementary DNA (cDNA), or directly, in the form of transcript-RNA in budding yeast. We found that transfer of genetic information from RNA to DNA occurs with an endogenous generic transcript in cis, and is thus a more common mechanism than previously anticipated. With the advent of CRISPR RNA-guided DNA endonuclease enzymes, there is marked interest in understanding the pathways to facilitate accurate genome engineering events. While ribonucleases (RNases) H1 and H2 block DSB repair by RNA, the recombination protein Rad52 is a key factor for this repair mechanism. DSB repair by RNA requires Rad52 but not the recombination protein Rad51, RecA homolog, or Rad59, which has homology with the yRad52 N-terminal domain (NTD). Upon overexpression of yRad52,yeast or hRad52 NTD, we observed a significant increase in the frequency of DSB repair by RNA. A 68-fold increase was obtained when hRad52 NTD was expressed in cells defective for RNase H function that was lacking the yeast RAD52 gene, indicating that hRad52 could catalyze DSB repair by RNA also in human cells. Moreover, in the absence of SAE2 or EXO1 genes, which are important for DNA end resection, the frequency of DSB repair by RNA was either increased or not changed, respectively. These results support an RNA-dependent mechanism of DSB repair mediated by Rad52 that catalyzes a reaction in which RNA invades a broken double-stranded DNA in conditions of limited end resection. Our results suggest that transcript RNA, like non-coding RNA, may have a significant role in genome stability and genome modification, much more prominent than previously anticipated.

Biography

Francesca Storici received her PhD in Molecular Genetics from the International School of Advanced Studiein Trieste, Italy (1998). She was a postdoctoral fellow at the National Institute of Environmental and Health Sciences (NIEHS, NIH), NC till 2007, and then research assistant professor at the Gene Therapy Center of the University of North Carolina at Chapel Hill, NC. She joined the Georgia Institute of Technology as an assistant professor in 2007, and became Distinguished Cancer Scientist of the Georgia.Research Alliance. In 2013, she was promoted to Associate Professor with tenure. In 2016, she became Howard Hughes Medical Institute Faculty Scholar. Just recently, she was promoted to Full Professor. Her research is DNA damage, repair and gene editing.

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Hamutal Meiri

Director of Exploitation ASPRE Consortium and CEO TeleMarpeh Tel Aviv, Israel

ASPRE model for prediction and prevention of preeclampsia and molecular approach for a personalized diagnosis with an attempt for prevention in an in-vitro model with CRISPER/Cas9

Preeclampsia (PE) affects 2-8% of pregnant women and is a major cause of short and long-term maternal and perinatal morbidity and mortality. ASPRE (Combining first trimester risk prediction and evidence based prevention by low dose aspirin) has shown a 75% PE risk prediction by history, biochemical and biophysical markers at 10% false positive rate, identifying 10% of the high risk population of pregnant women. Aspirin provided to the high risk group from the first trimester prevents 62% of preterm preeclampsia (<37 weeks gestation), 89% of preeclampsia <32 weeks, and reduced by 67% the duration of stay and cost of NICU. Yet 25% of preterm cases remained un-identified and term PE are detected in only 44% of the cases. Placental protein 13 (PP13) preeclampsia is a placental specific protein that can be detected in the maternal blood from the 5th gestation week. Reduced PP13 RNA and low first trimester maternal blood level are PE biomarkers. The protein was found to prime the maternal pregnancy vascular system to pregnancy by expanding the pregnancy veins and arteries thus enabling the increase in blood flow and the supply of nutrients and oxygen to the placenta and the fetus. The effect is mediated by the eNOS system providing Oxygen and the prostaglandin system responsible for vessels vasodilation in the first trimester of pregnancy. Low PP13 will lead to insufficient blood and oxygen supply to the pregnancy. The low PP13 is derived of molecular polymorphism of the PP13 proteins with -98A/A promotor variant associated with low PP13 and high risk to term PE, especially among obese women. The 221 thymidine deletion generates a truncated variant is associated with a shorter PP13 and high risk for very sever early preeclampsia associated with fetal and mother mortality. Both mutated variants are more prevailed in women of African origin and may account in part to the higher PPE prevalence in Africa. The truncated variant fails to prime the blood vessels expansion. Using the Crispr/Cas9 system we have generated the thymidine 221 deletion and could generate its repair. The truncated mutation reduces significantly PP13 expression and blood vessels expansion. Mutation repairs by this system- renew both effect. This approach has the potential for major reduction in death and handicap for mothers and babies.

Biography

Hamutal Meiri holds a PhD in Neurobiology from the Hebrew University of Jerusalem (1979) and a MBA degree from Tel Aviv University, Recanati School of Business (1995). She was a faculty member in brain development in the medical schools of Tel Aviv University, Technion, and NYU (1982-1990), and a Visiting Professorship at Weil-Cornell Medical College, NY. In 1991 she was appointed to be the first Director of Israel National Committee of Biotech, and also served as the Research Advisor to UNESCO COBIOTECH Committee. In 1994-1999 she was the Consortium Director of Israel Chief Scientist Magnet program (Biotech). In 1996-1998 she was elected to be the Head of Israel Telemedicine Industry Forum, and also served as a consultant to the Scandinavians Prime Ministers on Telemedicine.

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