

2252nd Conference
Bio America & CRISPR-2018



24TH BIOTECHNOLOGY CONGRESS: RESEARCH & INNOVATIONS

Annual Congress on
&
CRISPR CAS9 TECHNOLOGY AND GENETIC ENGINEERING

October 24-25, 2018 | Boston, USA

Poster Presentations

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Measuring and monitoring structural variation associated with gene editing using directional genomic hybridization (dGH) and automated image processing

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A number of widely used gene editing techniques, including CRISPR/Cas9, ZFNs, TALENs, and meganucleases rely on directed double-strand breaks and endogenous DNA repair mechanisms. Between repair mechanism failure and cellular damage induced by harsh editing system conditions, genomic structural changes are unavoidable. Therefore, it is critical to utilize techniques for the discovery and quantitation of genomic structural rearrangements in populations of cells both before and after editing. While individual errors are rare, even low prevalence errors or off-target effects pose risks to patients and require rigorous quantification and control for therapeutic applications. Directional Genomic Hybridization™ (dGH™) is a highly precise cytogenetic technique, enabling the direct visualization of genomic structural rearrangements of 5kb or less. Using the reference genome, dGH probes are designed against normal sequence and produced using single-stranded fluorescently labeled DNA. dGH probes are hybridized to prepared metaphase chromosomes and imaged, with a simple, accessible method. Structural variations from the reference genome are then easily visualized from the resulting signal. Assessment of cell lines or patient samples before and after gene editing elucidates the effects of the editing process on the desired edit site and identifies any off-target effects occurring above the baseline rate of the pre-edit sample. In this poster, we illustrate how dGH is used to discover and detect structural rearrangements missed by NGS and other methods that use pooled DNA, precisely detecting the prevalence of multiple and variable rearrangements occurring on a cell by cell basis. We describe our progress toward automated image analysis in control and edited cell populations. Process development such as AI-based image analysis and scoring will be presented.

Biography

KromaTiD provides innovative solutions for the discovery, detection and quantification of genomic structural variations. With our dGH™ platform, we are able to observe gene editing associated rearrangements in their chromosomal structural context. The data is complementary to sequencing and our assays provide researchers and innovators in gene editing with an additional dimension of data to support optimization of process variables, quantitation of structural offtarget effects, and tracking of durable genomic changes over time.

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Generation of transgenic chimeric ducks using blastoderm cell transfer CRISPR/Cas9-mediated gene insertion into the duck genome

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Statement of the Problem: The ability to culture and genetically modify embryonic cells changed developmental biology. The production of transgenic birds has increasing applications in biotechnology. There are various methods of birds transgenesis and the production of germline chimeras. The technique of injection DNA under the germinal disk could be used as one of the effective and often used methods. Weak reproductive ability and low survival rate of the produced chimeras are the main disadvantages of this method.

Methodology & Theoretical Orientation: In order to produce germline chimeras, the embryos of the Shan partridge duck were used as recipients, and blastodermal embryos of Shaoxing ducks were used as donors. Recipients were sterilized using ultraviolet light irradiation. The isolated blastodermal donor cells were transfected with the DNA-construction (CRISPR/Cas9-mediated gene insertion into the duck genome) with lipofectamine which was inoculated under embryo of the of the recipient eggs.

Findings: Survival rate of recipient embryos following transfected was 6.98% (19/272). After hatching, eleven female and eight male alive birds (3 % of the manipulated embryos) were obtained. Five of eleven female founders and five of eight male founders carried the transgene construct, and the transgenic bird production efficiency was 44.4% (from survive). Among the five male founders, only one (number 28) was most fertile. It produced 34 descendants (18 females and 14 males), of which two sons and nine daughters were transgenic (32.2%). Thus, the obtained data indicates that this technique the positive outlook on usage CRISPR/Cas9-mediated gene insertion into the duck genome of the Shan partridge and Shaoxing duck model for creating transgenic ducks and should be useful in developmental studies and may facilitate the production of transgenic poultry as the exogenous DNA was successfully inserted into the duck genome.

This study was supported by the Earmarked Fund for National Waterfowl-industry Technology Research System (CARS-42-06) and the Zhejiang Major Scientific and Technological Project of Agricultural (livestock's) Breeding (grant number 2016C02054-12).

Biography

Mariia Doroshenko is a doctor of veterinary medicine and a PhD student at the Department of Genetics, Breeding and Biotechnology of Animals at the National University of Life and Environmental Sciences of Ukraine. Her work is based on gene polymorphism in breeding. She has engaged in genetics and breeding in poultry work for more than two years.

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Zebrafish as genetic model for investigating the role of BCL6A and its regulation via STAT5

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Introduction: BCL6A has been recognized as an important transcription factor in the control of normal B cell development, as well as its disruption in B cell lymphoma, but with emerging roles in the development and function of other cell populations. A key aspect of BCL6A function is its transcriptional regulation by STAT5, which is activated by a number of cytokines, while there is evidence that BCL6A modifies STAT5 target gene regulation.

Methods: Bioinformatic analysis of zebrafish gene databases using tBLAST with human BCL6A and BCL6B sequences and alignment of the human BCL6A and zebrafish BCL6A proteins. The embryonic expression pattern of zebrafish BCL6A and regulation of BCL6A expression by STAT5, wild-type, *stat5.1^{-/-}* and *stat5.2^{-/-}* embryos were investigated by whole-mount *in situ* hybridization. The zebrafish BCL6A gene was targeted using genome editing with CRISPR/Cas9 designed to a region of exon 3 encoding the BTB/POZ domain to generate a BCL6A knockout zebrafish and used to analyze the growth and survival phenotype of the knockout. Additionally, Lymphopoiesis and macrophages activity was investigated through wound assay and immune challenge assay.

Results: Identification of zebrafish BCL6A with conserved structure and regulation, encoding a protein with high identity to human BCL6A. Ablation of BCL6A has shown severe retardation in growth, development, and survival of zebrafish.

Conclusion: Zebrafish represent an ideal model for investigating the BCL6A role in hematopoiesis and immunity.

Biography

Farooq Almohaisen was born in Iraq in 1982. He received the BVMS and MScM, degrees from the University of Basra in Iraq in 2005 and 2010, respectively. He joined the Southern Technical University in 2010 where he currently lectures. He started his PhD degree at the School of Medicine at Deakin University, Australia in 2014. His main work focused on the role of B cell lymphoma 6A protein (BCL6A) in zebrafish. He used CRISPR/Cas9 in editing BCL6A gene and investigate the *bcl6a* knockout on the immune system, growth and survival in zebrafish.

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Genome editing in microalga *Chlamydomonas reinhardtii* via CRISPR/Cas9

Irina Sizova

Humboldt University of Berlin, Germany

Statement of the Problem: It was found that the genome of a popular model organism, single-celled microalga *Chlamydomonas reinhardtii* encodes at least 18 sensory photoreceptors and functions of many of them are not completely characterized or unknown. We developed the efficient CRISPR/Cas9 based gene inactivation protocol (Greiner et al.2017) and disrupted 11 non-selectable photoreceptor genes. By sequencing of mutated genes, we found that precise repair of Cas9 induced double-stranded breaks (DSBs) through homologous recombination with supplied donor DNA was a rare event and mutated clones contained various DNA modifications of the target site. For further structure-functional investigation of photoreceptors and other proteins, it is required to generate predefined amino acid substitutions and insertions of fluorescent tags at their native genomic locus. The purpose of this study is to better understand DNA repair pathways and increase the efficiency, predictability, and fidelity of Cas9-induced site-directed mutagenesis in *Chlamydomonas*.

Methodology & Theoretical Orientation: We generated and characterized mutants on DNA repair pathways containing different ku80, ku70, polQ null alleles and used them as recipients for targeted mutagenesis with CRISPR endonucleases, including SpCas9, SaCas9 or LbCpf1, and various donor DNA substrates.

Findings: Inactivation of the POLQ gene resulted in a dramatic decrease of the rate of occasional and targeted DNA insertions into the nuclear DNA and the high sensitivity to the DNA damaging agents zeocin and MMS. Oppositely, all ku80 and ku70 mutants demonstrated the rate of occasional and targeted DNA insertions, spectra of targeted mutations and zeocin and MMS sensitivity similar to the wild-type cells.

Conclusion & Significance: POLQ could be responsible for the most of occasional and targeted insertions of DNA fragments into *Chlamydomonas* nuclear genome. Inactivation of KU80, Ku70 or POLQ does not increase predictability and fidelity of Cas9-induced mutagenesis in *Chlamydomonas*.

Biography

Irina Sizova has her expertise in evaluation and passion in genome editing in *Chlamydomonas*. Earlier, together with colleagues, she designed a resistance marker for paromomycin, which is now one of the most popular markers for the transformation of *Chlamydomonas*. Together with colleagues of Humboldt University of Berlin she has created or adapted a number of methods for site-directed modification of *Chlamydomonas* nuclear genome, including the use of single-stranded DNA vectors, zinc-finger nuclease and the system CRISPR/Cas9.

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Strategies for controlling off-target effects and biological variations in CRISPR/Cas9 genome editing experiments

Michelle Kimberland
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The CRISPR/Cas9 system has enabled efficient modification of genes in a variety of cellular systems for studying phenotypic effects of genetic perturbations. However, various levels of off-target effects (OTEs) have been reported. It can be difficult to conclusively determine that the observed phenotypic changes are in fact due to the intended modification of the target gene and not from unintended mutations elsewhere in the genome. In addition, biological variations observed within cultured cells can also confound results and need to be addressed. In this poster, designing and experimental strategies for minimizing and controlling OTEs and biological variations in CRISPR genome editing experiments are summarized, together with orthogonal approaches used to confirm on-target KO effects.

Biography

Michelle Kimberland has her background experience in molecular biology and genetics. She has worked on a variety of diseases/disease areas including hemophilia A, hepatitis C viral replication and womens' health issues. She is currently working at GlaxoSmithKline where her work is focused on genome editing for target selection, target validation and phenotypic assay development.

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Growth and yield of *Pisum sativum* L. (pea) in response to bio-fertilizer produced from *Rhizobium* species isolated from soya bean root nodules

Janet U Itelima

University of Jos, Nigeria

Statement of Problem: The indiscriminate use of chemical fertilizers to increase the soil nutrients and the use of pesticides is one major problem facing crop farming. Hence, the use of bio-fertilizers can be a very good complementary to the chemical fertilizers as they not only promote crop growth and yield but also maintain soil health for sustainable agriculture. The growth and yield of pea (*Pisum sativum* L.) in response to bio-fertilizer produced from *Rhizobium* species using poultry droppings and earthworm casts as carrier materials were evaluated.

Methodology & Theoretical Orientation: Soya bean (*Glycine max* L.) was cultivated to obtain the root nodules for the isolation of *Rhizobium* species. The nodules were sterilized, crushed, serial dilutions prepared, inoculated on Yeast Extract Mannitol Agar (YEMA) media and incubated at 28°C. The pure culture of *Rhizobium* was isolated, mass-produced and then mixed with sterile carrier materials (poultry droppings and earthworm casts), each for application unto the experimental crops. The *Rhizobium* broth and carrier materials were mixed in the ratio of 2 liters to 100kg. Analyses of experimental soil, poultry droppings, and earthworm casts were carried out to determine their physicochemical properties. Four treatments were replicated four times and arranged in a Complete Randomized Block Design (CRBD). Ten kilograms (10kg) of the fertilizer types each was applied to the cultivated ridges in plots A, B, and C Plot D (control) was not treated with fertilizer. Plant growth and yield parameters of pea grown on soil amended with the bio-fertilizers (of different carriers), inorganic fertilizers and the control were measured and compared.

Findings: The results showed an improvement in the growth and yield parameters of *Pisum sativum* (pea) that received *Rhizobium* bio-fertilizer over the control. There was a significant difference at ($p < 0.05$) in the growth and yield parameters of a pea in relation to fertilizer treatments. The highest improvement in the growth and yield of pea was observed in bio-fertilizer amended with poultry droppings, while the control had the lowest. Plants treated with inorganic fertilizer had a mean value of 1.84t/ha while the control gave the least yield of 1.25t/ha.

Conclusion & Significance: The outcome of this study is important in that farmers can fall back on *Rhizobium* bio-fertilizer for the cultivation of pea since the inorganic fertilizers are very expensive such that most poor farmers cannot afford them.

Biography

Janet Uchechukwu Itelima has her expertise in Applied Microbiology and passion in research related to Applied Microbiology, Biotechnology, and Plant Science, lecturing, and community services. She has obtained her PhD and currently an Associate Professor of Applied Microbiology. She is an academic staff of the Department of Plant Science and Technology, Applied Microbiology Unit, Faculty of Natural Sciences University of Jos, Nigeria. She has published 40 papers both nationally and internationally. She has also written two books. She is deeply involved in motivating students on how to obtain academic excellence. She has attended workshops and conferences both nationally and internationally, where she presented papers, chaired sessions and served in the advisory committee. She has recently been to the United States of America where she attended three conferences and also presented papers.

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Extracellular vesicle/prodrug-mediated specific treatment of HER2+ve breast cancer xenografts in mice

AC Matin

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This talk is concerned with therapeutic uses of EVs (exosomes) and deals with gene-delivered enzyme prodrug therapy (GDEPT), which promises to confine drug generation to the tumor at a high concentration and mitigating off-target effects. A new prodrug [6-chloro-9-nitro-5-oxo-5Hbenzo-(a)-phenoxazine (CNOB)] and the Escherichia coli enzyme HChrR6 that we discovered, and improved, and our recent success in specifically targeting it to implanted orthotopic HER2+ve breast cancer (BC) tumors in mice will be discussed; HChrR6 converts the harmless CNOB to the highly cytotoxic drug, 9-p amino-6-chloro-5H-benzo[a]phenoxazine-5-one (MCHB). As mRNA is superior to DNA for gene delivery and EVs less subject to immune rejection, mRNA encoding the HChrR6 enzyme-loaded EVs that displayed an anti-HER2 scFv antibody (on a chimeric protein termed EVHB) were used. These "EXO-DEPT" EVs—but not the non-directed, non-mRNA containing EVs—imparted CNOB activating capability specifically on the HER2+ve BT474 cells *in vitro* and caused the complete arrest of implanted orthotopic BT474 tumors in immunocompromised mice. This is the first time that foreign functional mRNA was successfully delivered using EVs. As the anti-HER2 scFv in EVHB can be replaced by other targeting moieties, this approach can be employed to treat any disease overexpressing a specific marker. *In vivo* ablation of the tumor is a potent method for stimulating robust HER2-specific adaptive T- and B-cell responses. Results will be presented of ongoing work in collaboration with Dr Kim Lyerly (Duke University) on the reinforcing effect of this response on the efficacy of the EXODEPT/CNOB regimen. A BC mouse model driven by an oncogenic form of human HER2 (HER2 Δ 16) is being used. It relies on mammary-specific expression of HER2 Δ 16, leading to aggressive breast tumor development within weeks of induction, with strong central tolerance to the HER2 Δ 16 epitope. Measurements underway involve HER2-specific systemic T-cell responses (T-cell ELISPOT assays from harvested splenocytes; assessment of cytokine flow), tumor regression and mouse survival.

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Biotechnological approaches as alternatives for exploiting the production of important secondary metabolites- *Rubia tinctorum* L. cell, tissue and organ culture: A case study

Ana Rosu

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Since early times mankind exploited the genetic diversity of the plant kingdom as a major source of an array of secondary products, widely employed as pigments, food additives, pharmaceuticals, cosmetics and agrochemicals of economic importance. Madder (*Rubia tinctorum* L.) root has been used for dyeing textiles in many parts of the world and over the centuries was an important export product throughout Europe. The natural dye components are anthraquinones, especially alizarin, present in the madder root mainly as its glycoside—the ruberythric acid. At the end of the 19th century, the use of madder for dyeing declined due to production at large scale of synthetic alizarin. At present, as the production of synthetic alizarin involves increasing costs and gives polluting side effects, the use of natural dyes became more popular and revitalizing of madder as an industrial crop is reconsidered. Besides being the source of the valuable red dye, madder components are reported to exhibit various pharmacological activities, including anticancer, antimalarial, antibacterial, antifungal and antioxidant activities. Biotechnological approaches, specifically plant cell, tissue and organ culture, play a recognized vital role in the search for alternatives to production and accumulation of valuable compounds. Our preliminary studies focused on defining the conditions for obtaining dependable in vitro cell biomass, resulting in the development of rapidly-growing, long-term callus cultures. Though the detection of alizarin and of other anthraquinones in the madder callus cells was not the purpose of this stage of our experiments, some preliminary testing with the coloring capacity of the madder callus cells on wool fibers were performed, proving that madder cells in culture retain complete genetic information, being chemically totipotent like the mother plant in nature.

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Analysis of risk in CVD via human genetics and biomedical equipment

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Statement of the Problem: In applied Biomedical Engineering discipline of human factors analysis is a complex and evolving study in cardiac surgery. Some realistic effort to reduce human error arose with the observational nature of human factors engineering we can take to analyze Risk Theory in cardiac surgery. According to a report from ECRI, here are the 10 riskiest areas which we have to analyze. Infusion errors which may be deadly to Patient. One big issue that can slip through the cracks is “IV free flow”. Secondly, inadequate cleaning of complex reusable instruments which recent rash infections attributed to the reuse of instruments after sanitizing and staff should be regularly reminded of the correct cleaning protocol. Third missed ventilator alarming Robotic Surgery may also be associated with higher costs and additional risks. Risk Analysis in CVD can be monitor through Bayesian Analysis to integrate independent dataset, Bayes factor as a function of SNP in the CHD population. To test the robustness of Bayesian analysis, we examine two tests of the sensitivity, namely to low significance data sets. we exclude the data sets with comparatively small sample sizes, we also exclude large Bayes Factor.

Methodology: We use a Bayesian spatial model to estimate CVD mortality by ward, sex and age group for the period of 2012-2018 in Pakistan. The number of deaths in each ward the period-sex-age group was specified using a Poisson model. The Poisson model estimates mortality in each ward.

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Phenolic derivative of polyglyceric acid from medicinal plants its synthetis monomer and their anticancer efficacy

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According to data of different techniques of NMR spectroscopy ¹³C, ¹H NMR, 2D heteronuclear ¹H/¹³C HSQC, 1D NOE and 2D DOSY experiments the main chemical constituent of high molecular preparations from medicinal plants of different species of two genera *Symphytum* and *Anchusa* (*Boraginaceae* family) *Symphytum asperum*, *S. Caucasicum* (*caucasicum endemic*), *S.grandiflorum* (*Georgian endemic*), *S. officinale* and *Anchusa italica* was found to be poly[oxy-1-carboxy-2-(3,4-dihydroxyphenyl) ethylene] or poly[3-(3,4-dihydroxyphenyl)glyceric acid] (PDPGA). The polyoxyethylene chain is the backbone of this polymer molecule and 3,4-dihydroxyphenyl and carboxyl groups are regular substituents at two carbon atoms in the chain. The repeating unit of this regular polymer is 3-(3,4-dihydroxyphenyl)glyceric acid residue. In order to compare biological properties of natural polymer with its synthetic analogs, racemic and pure enantiomeric forms of PDPGA, as well as a methylated analog of PDPGA, were synthesized. The racemic monomer rac 2,3-dihydroxy-3-(3,4-dihydroxy-phenyl)propionic acid (DDPPA) and its pure enantiomers (+)-(2R,3S)- DDPPA] and (-)-(2S,3R)-DDPPA] were synthesized via sharpless asymmetric dihydroxylation of trans-caffeic acid derivatives using an potassium osmate catalyst, a stoichiometric oxidant N-methyl morpholine-N-oxide and enantiocomplementary catalysts cinchona alkaloid derivatives (DHQ)2-PHAL and (DHQD)2-PHA as chiral auxiliaries. Methylated PDPGA was obtained via ring-opening polymerization of 2-methoxycarbonyl-3-(3,4-dimethoxyphenyl)oxirane using a cationic initiator. PDPGA is endowed with intriguing pharmacological activities as anticomplementary, antioxidant, anti-inflammatory, burn and wound healing and anticancer properties. PDPGA and its synthetic monomer exerted anticancer activity *in vitro* and *in vivo* against androgen-dependent and -independent human prostate cancer (PCA) cells via targeting androgen receptor, arrest and apoptosis without any toxicity, together with a strong decrease in prostate specific antigen level in plasma. However, the anticancer efficacy of PDPGA against human PCA cells is more compared to its synthetic monomer. Methylated PDPGA did not show any activity against PCA. Overall, this study identifies PDPGA as a potent agent against PCA without any toxicity and supports its clinical application.

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Super high oleic safflower: Australia's new high value broad acre crop producing industrial grade plant-derived oil

Carl M Ramage
Rautaki Solutions, Australia

Plant-derived oils are mixtures of saturated, monounsaturated and polyunsaturated fatty acids in ratios that are less than ideal for industrial uses that often demand high purity in feedstock composition. Large volumes of crude vegetable oil containing near-pure levels of oleic acid have long been considered a desirable industrial feedstock, offering unique physical and chemical properties for oleochemical purposes. Safflower (*Carthamus tinctorius* L.) seed produces oil that predominantly contain monounsaturated fatty acid (C18:1; oleic acid) and polyunsaturated fatty acid (C18:2; linoleic acid). While both have commercial uses, it is the valuable oleic acid that is used as a replacement to petroleum-based precursors in the manufacture of plastics, lubricants and cosmetics, etc. Traditional breeding programs have developed safflower seed with oleic acid levels in the range of 75–85%, and are the highest purity sources of oleic acid in any oilseed. However, like other oilseeds, the remaining linoleic acid component, at 12-18%, is not desirable for industrial use because it is unstable and difficult to remove during oil processing. Therefore, it is desirable to develop a safflower seed that accumulates high oleic acid (C18:1), but contains very low linoleic acid (C18:2) content. Two genetically modified safflower events were developed by the Commonwealth Scientific Industrial Research Organisation (CSIRO) and are being commercialized by GO Resources Pty Ltd. The events contain a construct designed to down-regulate two safflower fatty acid biosynthesis genes. Down-regulation is achieved using RNAi technology and is targeted to the seed using a seed-specific promoter. Down-regulation of the two safflower genes leads to accumulation of 92% oleic acid (C18:1) and very low (less than 2%) linoleic acid (C18:2) in the seed, Super High Oleic Acid Safflower Oil (SHOSO). Details of the development and commercialization of this new GM crop in Australia will be presented.

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Plant biotechnology applied in pharmacological research and in problems of the agricultural sector in Mexico

Elsa Ventura Zapata

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Some problems are described in the areas of agriculture, health and social of Mexico and their solution through plant biotechnology. The State of Morelos is a producer of "Morelos" rice; It enjoys national and international prestige for its industrial and culinary characteristics, however, it has a high cost of cultivation and few profits for the producer. Because of this, it was developed by cultivating anthers, a variety with high grain quality, to be placed at a better price in specific market niches. Mexico ranks first as an exporter of papaya. This culture presents virosis problems, for this reason, the technique of cultivating anthers to generate resistant varieties is being applied. In the Municipality of Tlayacapan Edo. De Morelos venerate each year the "child God", for it adorns the altars with the species *Agave dasyliroides* Jacobi & Bouche, which grow literally in the rocks. It is currently overexploited, so the Municipal President requested its *in vitro* propagation to repopulate the natural habitat. 5000 plants were generated with which the species continued to be multiplied. The low percentage of survival during the acclimatization of plants propagated *in vitro* motivated to develop a prototype to reduce mortality; this was reduced to 0.0.e. Taxol is a highly demanded anti-carcinogen; hence the importance of increasing its yield in *in vitro* cultures; a process was patented to increase the production of this substance in cell cultures of *Taxus globosa* Schtdl. Bioprospecting projects are currently being developed in order to take advantage of wild resources for food and medicinal purposes. Methods of micropropagation and hydroponic cultivation of different plants of Silvestre origin have been established.

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Glioblastoma multiforme and Indian medicinal plants

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Glioblastoma Multiforme (GBM), the most aggressive, malignant and common brain tumor with a patient survival rate of fewer than 15 months. Till now, cure as well as, proper standardized treatment of GBM, which can enhance the survival rate, is stagnant. Now scientists are looking back on herbal regimes, worldwide and are attempting to increase the survival and quality of life of GBM patients. Herbal approach to treating various chronic disorders is going on from last many decades. Studies over the therapeutic application of plants in cancer prove to be an effective approach towards it. The appropriate ones used in GBM treatment are mentioned below:

- *Curcuma* species, widely used ingredient in Indian cuisine (haldi), possess various medicinal benefits, from antioxidant property to cancer inhibition. Specifically, in GBM patients, it has been shown to induce autophagy, apoptosis by targeting death signaling pathways (G2/M).
- Ashwagandha (*Withania somnifera*), traditionally used the herb in Ayurveda, known for its antioxidant, immune-stimulant, analgesic, anti-cancerous, and neuro-regeneration etc. properties. In glioblastoma cells, it causes apoptosis, growth arrest and also downregulates the proinflammatory cytokines expression suggests this herb as a potent anti-cancerous approach to GBM.
- One more herb i.e. black soybean, edible bean widely grown for its benefits. In glioma cells, they have been reported responsible for the caspase-mediated cell death and anti-invasive activity of its saponin content. Also, anthocyanins present in soybean promotes autophagy by silencing the atg5 expression which against the oxygen-glucose deprived stress induces cytotoxicity in cells.

So, in the present paper, various Indian herbs have been reviewed and described for their use in brain cancer. The paper includes their pharmacological uses and their targets which are found to be useful for the treatment of brain cancer.

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