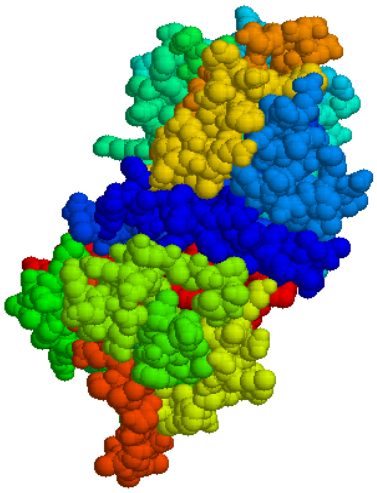

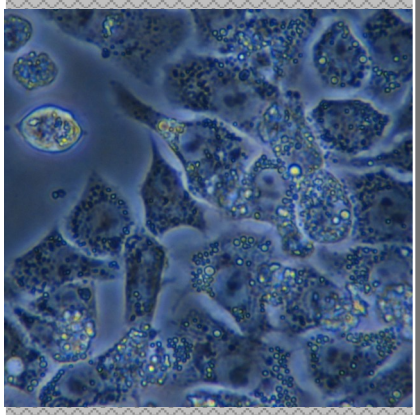

EMGEN Newsletter

Vol. 6, Issue 6

IN THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMGEN) was created in 2004 with collaboration of representatives of selected centers of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO. Sponsored by Iran Biotechnology Development Council.

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ANTIBODY INFORMATICS

Modern developments in lab techniques permit scientists to quickly create a huge amount of data utilizing a variety of molecular and biological approaches. The data-driven information should be altered to model-based information. Pharmaceutical corporations require to handle big biological data sets because molecular biology is importantly engaged in drug development, discovery and manufacturing. However, the expense engaged in catching up with this fast growth inhibits any single company from adapting to these huge biological data sets rapidly and effectively. Some efforts toward precompetitive collaborations are underway. There are many more informatics techniques accessible for the study of small molecule therapeutics than for the antibody drug discovery procedure. Here, some different view of "antibody informatics" is demonstrated.

***In silico* antibody design**: **Rational approaches to humanize antibodies**

For nonhuman antibody libraries, rational methods to humanize antibodies have been the principal approaches for *in silico* projects. They are extensively practiced for decreasing the danger of immunogenicity. Practical approaches to humanize antibodies are characterized by creating some types of the proteins and evaluating their binding or any other desired characters. If the engineered types do not produce the anticipated results, a novel cycle of engineering and binding evaluation is initiated. Below, the different rational methods to humanize antibodies are explained.

1. CDR grafting

There are three key decision-making points of a typical complementarity-determining region (CDR) grafting method. These decision-making points contain: definition of fragments determining the specificity of the donor antibody and therefore target for grafting, source of human sequences to be used as a frame (FR) donors, and picking of residues outside of the fragment defining the specificity and hence a target for back mutation to improve or restore the affinity of the humanized antibody.

2. Resurfacing

In the early 1990's, resurfacing introduced as an alternative protocol to CDR grafting. This approach shares with CDR grafting the two first decision-making points of the design cycle. However, different from CDR grafting, resurfacing method keeps the non-exposed residues of the nonhuman antibody. Only surface residues in the nonhuman antibody are replaced to human residues.

3. Super humanization

CDR grafting depends on the FR association of the human and nonhuman sequences. Superhumanization is a substitute paradigm based on the association of CDRs, in which FR homology is unrelated. The technique includes association of the nonhuman sequence with the practical human germ-line gene collection. Those genes encoding the identical or sharply related canonical structures of the rodent gene sequences are chosen. Subsequently, within the gene sequences sharing the canonical structures with the nonhuman antibody, those with greater homology within the CDRs are selected as FR donors. Next, the nonhuman CDRs are grafted onto these FRs.

4. Human String Content Optimization

In 2007, a novel technique was presented for antibody humanization according to a metric of antibody humanness named Human String Content (HSC). This technique associates the murine sequence with the collection of human germline gene sequences and the dissimilarities are scored as the HSC. Next, the target sequence is humanized by maximizing its HSC rather than applying a global identity measure to create multiple different humanized variants.

Affinity maturation and elimination of physicochemical problems

1. Affinity improvement by somatic mutations and computational design

Antibodies can emerge in a brief time in reply to antigens, so that they are more precise to their antigens and have higher affinity, essentially by improving the complement of the antigen- antibody interfaces. Somatic mutations may appear not only in the antigen-binding area, but also in the framework area, which antigens routinely do not contact physically. Computational methods have a significant impress in finding out how these mutations enhance binding attraction. Newly, these methods have been applied to predict novel mutations to improve affinity or specificity. According to the study of a large number of sequences, trends have been examined in amino acid replacements during the somatic maturation process. Particularly, utilizing a gene-fitting process with codon possibility tables, mutation possibilities were examined in 23116 heavy chains and 11095 light chains. By comparing the possibilities with the conventional BLOSUM matrix, it was deduced that amino acid replacements in somatic mutation procedure have almost similar trends to those seen in protein evolution. It was shown that, mutations tend to appear in antibody-antigen interfaces and in exposed superficial regions, rather than the framework area.

The inspection of alterations of amino acid arrangement in the antibody-antigen interface showed that the numbers of serine, tryptophan and tyrosine residues reduces but those of proline, histidine and phenylalanine increased through the maturation process. Several computational investigations have been conducted to study the relationship between somatic mutations and conformational flexibilities of antibodies. A reasonable hypothesis is that antibodies gain rigidity through maturation in order to expand binding affinity (by declining entropic losses upon binding) and to enhance specificity. Actually, crystal structures of antibodies accessible in the PDB are expected to acquire rigid conformations based on their sequence features. In details, the rigidification was hypothesized to take place within the entire variable domains rather than within only CDRs, by forming normal secondary structures, such as beta-turns and hydrogen bonds, or by forming residue contact networks during the maturation procedure. Thermodynamic studies and molecular dynamics (MD) dramaturgies validated that all residues in variable domains might engage in the rigidification rather than the limited number of CDR residues.

More recently, several germline and matured antibody constructions in the PDB were analyzed by the multi-constraint computational plan protocol with the Rosetta scoring function. Outcomes verified that native sequences of germline CDR-H3 are intended for conformational flexibility while those of matured ones are greatly optimized for single conformations. On the other hand, rigidification upon maturation is not common, since SPE-7, a matured anti hapten antibody, is recognized to undertake some various conformations in response to attaching dissimilar antigens. In addition, several studies of anti hapten antibodies have illustrated that quick maturation based on CDR rigidification tends to arrive at a moderate affinity while flexibilities in CDR-H3 may have a significant role in higher affinity maturation. To more correctly find out how somatic mutations control CDRs flexibility in nature, scientists analyzed a series of antibodies, whose crystal assemblies were solved both in germline and matured types, without their antigens in the crystal assemblies, utilizing MD simulations with an implicit solvent model. Even though antibodies can evolve *in vivo* via tendency maturation, discussions have showed that attaching tendencies and specificity could be more developed by utilizing artificial systems, for example computational protein designs and synthetic antibody libraries. Most of the present examples of computational antibody design came from antigen–antibody complex structures in the PDB, which the analysis can be named as re-design of antigen-antibody interfaces. Protein interface redesign depends on calculating free energy changes due to amino acid substitutions, which can be carried out by utilizing physics-based power arenas or knowledge-based capacities obtained from the structural database.

Alike comparative modeling approaches, re-design also needs suitable conformational search algorithms, such as dead-end elimination (DEE) and Monte Carlo (MC) searches, to examine conformations of modified constructions. In most investigations, due to computational restrictions, the protein spinal column has been stabilized through the processes. However, it has become probable to create specific spinal column pliability in computational protein sketch.

A dissimilar application of computational modeling is to find appropriate positions for experimental random mutagenesis, rather than relying on computational prediction to find beneficial mutations. In such a survey, scientists applied computational modeling, containing constrained antigen docking and MD simulations to pick desirable residues for random mutagenesis in CDR-H3 and CDR-L3 by phage display. This technique led to mature anti-gastrin antibodies with a 454-fold improvement in binding affinity compared to wild type based on six mutations.

2. Stability design and aggregation in antibodies

In antibody engineering, another challenging is the utilization of computational designs to anticipate aggregate -prone regions (APRs) from amino acid sequences and to design aggregate-resistant antibodies by inserting mutations in those regions. One mechanism of aggregation is the creation of amyloid fibrils, which are rich in beta-sheet structures, and have been engaged in diseases such as Alzheimer's disease. Experimental studies suggest that short fragments of a protein could simplify aggregate, and these are named as APRs. Currently, some computational techniques are accessible to anticipate APRs and calculate rates of aggregations, especially according to the sequence composition and on propensities as well as charge, hydrophobicity and secondary structure propensity. Empirical or phenomenological models have been utilized for evaluating the impact of mutations on the aggregation rate according to the experimental data. MD simulations have also been applied to complement and interpret experimental investigations of protein aggregation and amyloidosis. Aggregation of therapeutic proteins may cause problems with immunogenicity. Moreover, it has been realized that antibodies aggregate in high concentration during storage and in therapies. Many experimental studies carried out to survey antibody constancy and resistance to aggregation, mainly utilizing single-chain Fv fragments (scFv). Designed mutations for aggregation-resistant antibodies may induce the loss of desired affinities. In 2011, outcomes of a trial were applied to analyze APRs in an antibody. It is noteworthy that, while the outcomes of the computational approaches contain several false positives, the consensus of APR of those prediction tools is almost identical to the aggregation hot spots in the experiment.

This shows that computational tactics that integrate anticipation outcomes from several techniques can help to make better anticipation of APRs in antibodies. In 2011, MD simulations were performed with the aim of introducing a tactic for enhancing the thermal resistance of an anti-VEGF scFv antibody. The simulations represented that, during the elevating of the temperature, the dissociation of VL/VH domain interface of the antibody happens initially follows by unfolding of VL and VH domains. This finding shows that the stability of the VL/VH domain interface may control the stability of whole antibody structure. If this is accurate, then designing of the VL/VH domain interface is the basic selection for enhancing the thermal resistance of the antibody. The deep investigations of VL/VH domain interfaces could give direction for designing more stable antibodies.

3. Producing highly thermo resistant antibodies

In silico antibody manufacture can be applied to create extremely thermo-resilient antibodies by changing superficial residues to charged amino acids and it may be applied to enhance crosslinking efficiencies while presenting unusual amino acids in antibody CDRs. These methodologies require crystallization of the antibody/antigen complex. An alternative computational method for inclination increasing deprived of the crystal assembly is utilized by applying the physicochemical properties shared in epitope/paratope connections, which have been obtained from known 3D arrangements. This technique was used in antibody 4E11, a cross-reactive deactivating antibody to Dengue virus, in absence of crystal assembly, and a 450-fold improvement in inclination was obtained. This enhanced inclination and led to bigger neutralizing activity *in vitro*. It also led to potent antiviral activity in a murine model of DV challenge. Accurate and high quality antibody modeling are important goals when utilizing these techniques.

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COLD-PCR

PCR has become the key method in molecular analysis, with nearly every genetic examination that aims to determine DNA sequence differences incorporating PCR. As usually applied, PCR does not include an innate selectivity toward different mutant alleles; therefore, both mutant and non-mutant alleles are amplified with nearly equal efficacy. The duty of detecting and sequencing a variation in a PCR product falls on downstream analysis containing pyrosequencing, Sanger sequencing, MALDI-TOF mass spectrometry, HPLC, and RFLP test. Although it is reliable for screening common somatic mutations or germline, sequencing of unknown low -level mutations utilizing these strong techniques is still ambiguous. However, the importance of spotting these mutations is critical in a number of fields of medicine, comprising prenatal analysis, tumors and infections. COLD-PCR is an innovative scheme of PCR that favorably enriches 'minority alleles' from a mix of mutation-bearing and wild-type sequences, regardless of where an anonymous mutation locates. Therefore, COLD-PCR amplification of genomic DNA produces PCR products consisting of high percentages of mutated alleles, thus allowing their identification. Since PCR is a primary stage in approximately all forms of genetic assays, COLD-PCR produces a universal policy to increase the delicacy of all DNA variation-detection techniques.

COLD-PCR method

A single-nucleotide mismatch across a double stranded DNA sequence creates a little calculable change in the melting temperature (Tm) of that sequence. Based on the location of the mismatch and the milieu of the sequence, Tm changes of 0.2–1.5 °C are routine for sequences up to 200 base pairs (bp) long or longer. In 2008, scientists observed that for every DNA sequence, there is a crucial denaturation temperature (Tc) less than the Tm which PCR usefulness decreases suddenly and that the Tc is strongly based on the DNA sequence. DNA amplicons variants in a single nucleotide reproducibly have dissimilar amplification efficacies while the PCR denaturation temperature is set to Tc. This finding can be employed during PCR amplification for discriminating enrichment of minority alleles varying by one or more nucleotide at any position of a sequence. In this technique an intermediate annealing temperature is applied while PCR cycling to permit cross-hybridization of wild-type and mutant alleles. Next, hetero-duplexes which melt at lower temperatures than homo-duplexes are optionally denatured and intensified at Tc, while homo-duplexes stay double-stranded and do not intensify effectively. During the intensification, mutations at any position along the sequence are enriched by fixing the denaturation temperature to Tc.

A schematic diagram of COLD-PCR compared to conventional PCR is represented in the figure 1.

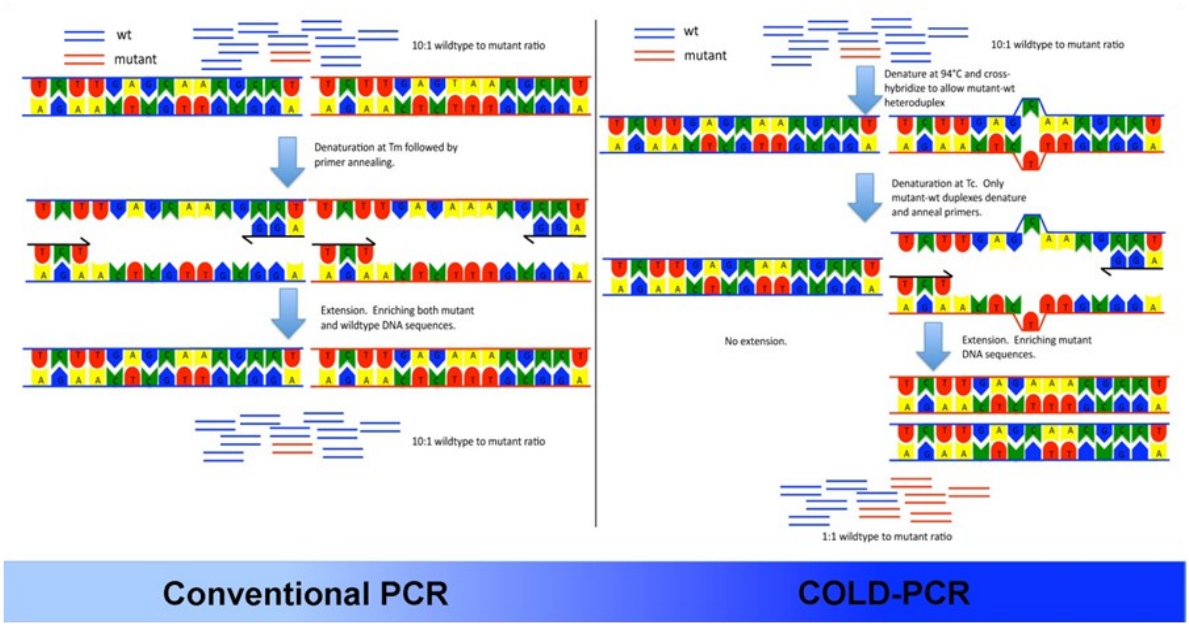

Figure 1: Regular PCR compared to COLD-PCR.

Keeping these rules in the mind, scientists introduced the following common protocol:

1. Denaturation step: DNA is denatured at a high temperature generally 94 °C.

2. Intermediate annealing step: an average annealing temperature is set that lets hybridization of mutant and wild-type allele DNA to each other. Since the mutant allele DNA forms a smaller proportion of DNA in the mixture, they will be more probably to create mismatches hetero-duplex DNA with the wild-type DNA.

3. Melting phase: the hetero-duplexes further melts at lesser temperatures. Therefore, they are selectively denatured at the Tc.

4. Primer annealing step: the homo-duplex DNA advantageously remains double stranded and is not accessible for primer annealing.

5. Amplification step: the DNA polymerase amplifies complementary to the template DNA. Since the hetero-duplex DNA is utilized as a template, a larger fraction of minor mutant DNA will be amplified and be accessible for following subsequent rounds of PCR.

Forms of COLD-PCR

1. Full COLD-PCR: Full COLD-PCR is same to the method described above. Five above mentioned steps are applied for each round of amplification.

2. Fast COLD-PCR: Fast COLD-PCR varies from Full COLD-PCR in that the denaturation and intermediate annealing steps are omitted.

Since in some conditions, the chosen intensification approach of the mutant DNA is so considerable that guaranteeing the generation of the mutant/wild-type hetero-duplex DNA is not acquired. Therefore, the denaturation can take place at the Tc, proceed to primer annealing, and finally polymerase associated extension. First three steps of the procedure are equal in each round of intensification. By using the lesser denaturation temperature, the reaction tends to generate products with the lesser Tm comprising the mutant alleles. Fast COLD-PCR gives quicker results because the shortened technique. Nevertheless, it is necessary to note that Full COLD-PCR is significant for amplification of all probable mutations in the initiating mixture of DNA. An improved form of Fast COLD-PCR is two-round COLD-PCR, in which through the second phase of Fast COLD-PCR nested primers are employed. In contrast to one-round Fast COLD-PCR, two-round COLD-PCR raises the accuracy of mutation finding.

COLD-PCR applications

1. RFLP

A RFLP leads to the slice of DNA for a particular mutation by a precise restriction enzyme that does not slice the wild-type DNA. In a research by utilizing a combination of wild-type and mutation comprising DNA intensified by conventional PCR or COLD-PCR, COLD-PCR preceding RFLP analysis was represented to improve the mutation determination by 10 to 20 fold.

2. Sanger sequencing

Sanger sequencing recently have been employed to assess the improvement of mutant DNA from a combination of 1:20 variant:wild-type DNA. The mutant DNA including a mutation was retrieved from a breast tumor cell line known to include p53 mutations. The analogy of Sanger sequencing chromatograms revealed that the mutant allele was enriched 13 fold while COLD-PCR was utilized compared to conventional PCR alone. This was discovered by the size of the peaks on the chromatogram at the different allele region. Moreover, COLD-PCR was employed to determine p53 mutations from lung-adenocarcinoma specimens.

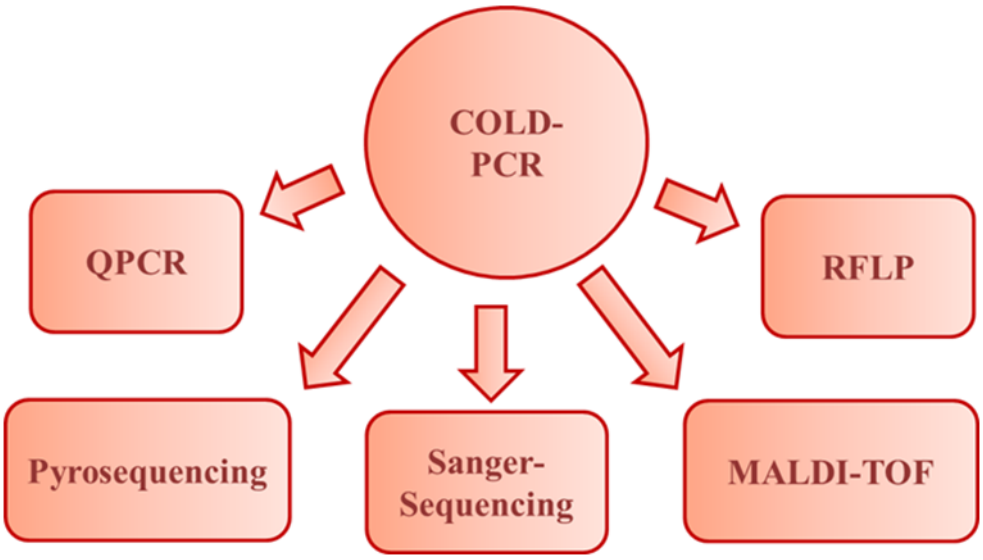

Figure 2: Downstream applications utilizing traditional PCR that can be replaced by COLD-PCR.

The research was capable to find out eight low level mutations that would probably have been lost during applying prevalent methods that don't enrich for mutant sequence DNA.

3. Pyrosequencing

Using of COLD-PCR in pyrosequencing was shown to be able of discovering mutations that had a prevalence of 0.5-1% of the samples applied. COLD-PCR was employed to determine KRAS and p53 mutations by pyrosequencing, and was represented to excel regular PCR in both cases.

4. MALDI-TOF

The research team that introduced COLD-PCR and utilized it to compare the sensitivity of traditional PCR for genotyping with direct Sanger sequencing, RFLP, and pyro-sequencing, also carried out a related effort by using MALDI-TOF as a downstream process for discovering mutations. The results showed that COLD-PCR could enrich variant sequences from a mixture of DNA by 10-100 folds. Besides, compared to the 5-10% low-level detection rate expected with traditional PCR, mutations with an initial prevalence of 0.1-0.5% would be detectable by COLD-PCR.

5. QPCR

COLD-PCR performance on a quantitative PCR apparatus, using TaqMan probes special for a mutation, was shown to enhance the measured diversities among wild-type and mutant samples.

Advantages of COLD-PCR

The single-step method is able to elevate both known and unknown minority alleles regarding the mutation type and location. COLD-PCR does not need any extra substances or particular machine. Thus, the cost is not raised. For the detection of mutations in a mixed sample, COLD-PCR acts better than regular PCR. It also does not considerably increase experiment run time compared to conventional PCR.

Disadvantages of COLD-PCR

During COLD-PCR, optimal Tc should be calculated for each amplicon. An additional stage should be added in contrast to regular PCR-based process. Accurate denaturation temperature must be regulated through PCR about ±0.3°C. A suitable crucial temperature that discriminates between wild-type and mutant DNA sequence may not be accessible. COLD-PCR is restricted to analyzing sequences less than nearly 200 bp. Overall mutation enrichment is variable and depends on DNA position and nucleotide substitution. There is no warranty that all low-level mutations will be suitably enriched.

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OPEN CHROMATIN PROFILING KEY TO IDENTIFYING LEUKEMIA CELLS OF ORIGIN

Each cancer begins with a single cell, and the Jackson Laboratory (JAX) scientists have discovered a whole-genome profiling of open chromatin as a reliable and exact way to recognize the kind of cell that results in a given case of leukemia, an important key to cancer diagnosis and treatment. "Identifying the cell of origin of cancer cells may give sight into tumor subtypes and probably prognostic and therapeutic advantage," says the lead author of the study Professor Trowbridge. "But existing approaches to detect cell of origin from mass tumor cell samples have been unfavorable." Trowbridge lab applied a murine model of acute myeloid leukemia (AML) driven by expression of MLL-AF9. MLL-AF9 is a fusion oncogene created by a chromosomal translocation among human chromosomes 9 and 11. To profile the open chromatin in these specific AML specimens and match them to open chromatin templates in normal cells, scholars cooperated with developers of computational methods to investigate gene translation containing chromatin assemblies. Eventually, they found gene expression templates and open chromatin signatures in AML specimens that can let progenitor cell of origin AML to be discriminated from stem cell-derived AML.

Reference: https://www.sciencedaily.com/releases/2016/07/160711092306.htm

DESIGNER VIRUS ACTS AS A MOLECULAR-LEVEL SWITCH

The ability to switch illness-causing genes on and off remains a vision for many scientists, physicians, and patients. A group of scientists has modified a virus to transmit the genetic materials to target nerve cells and tissue in the body. “In the lab, we utilized un-duplicable, weakened viruses that are described as modified adeno-associated viruses. We utilized these viruses to transmit genetic materials to vivid organisms affected by illness", said senior researcher, Dr. Hasan. "This method opens up a wide spectrum of options which may lead to cure and treat various illnesses in the future."

Reference: https://www.sciencedaily.com/releases/2016/07/160719110225.htm

YEAST, HUMAN CELLS AND BIOINFORMATICS HELP DEVELOP ONE-TWO PUNCH APPROACH TO PERSONALIZED CANCER THERAPY

In a study to increase the amount of tumor gene mutations that may be especially targeted with personalized medicine, researchers at the University of California and their co-workers searched composition of drugs and altered genes that jointly destroy tumor cells. Such compositions are anticipated to destroy tumor cells, which have mutations, but not normal cells, which do not. The study detected 172 new compositions that may build the way for novel tumor cures. "According to a patient's specific tumor mutation, oncologists can usually personalize tumor cure" mentioned chief scientist T. Ideker. "However, the broad majority of mutations are not actionable and knowing a patient has a special mutation doesn't signify there's an accessible cure that targets it. The aim of this research was to extend the number of mutations we can pair with an accurate cure."

The scientists initially applied yeast to survey 169,000 connections among genes that can be suppressed with medications speedily and reasonably, called "druggable" targets, and yeast types of humanoid tumor-silencing genes. Subsequently, they prioritized 21 medications for which the yeast druggable targets were engaged in the number of cells lethal interactions. They examined nominated medications individually for fatal relations with 112 various tumor-silencer gene mutations in humanoid tumor cells cultured in the lab. The scientists finished up with 172 medication-gene mutation compositions that favorably destroyed both human and yeast tumor cells.

Reference: https://www.sciencedaily.com/releases/2016/07/160721142801.htm

Book Alert

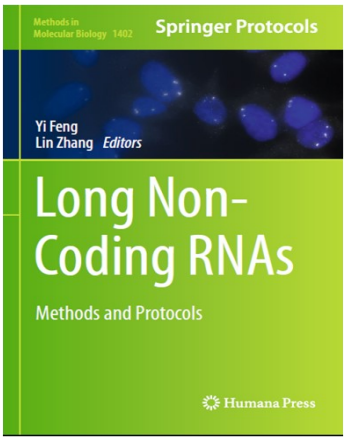
LONG NON-CODING RNAS

Publisher: Springer international publishing

Editors: Yi Feng and Lin Zhang

Publication date: 2016

ISBN: 978-1-4939-3376-1



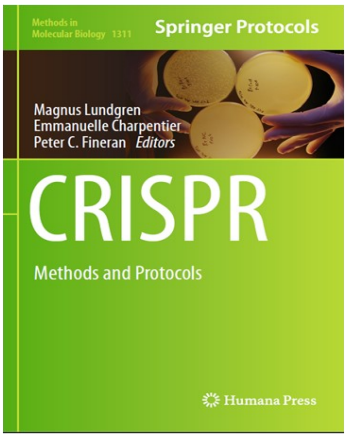
CRISPR

Publisher: Springer international publishing

Editors: Magnus Lundgren, Emmanuelle Charpentier and Peter C. Fineran

Publication date: 2015

ISBN: 978-1-4939-2686-2



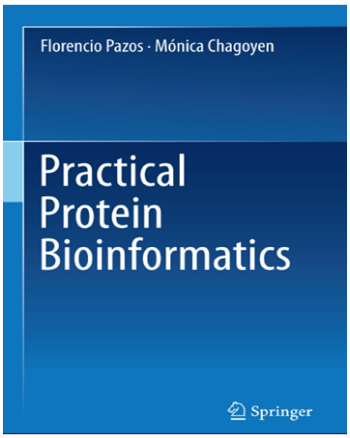
PRACTICAL PROTEIN BIOINFORMATICS

Publisher: Springer international publishing

Editors: Florencio Pazos and Monica Chagoyen

Publication date: 2015

ISBN: 978-3-319-12726-2



Announcements

http://www.genomeediting-congress.com/

http://www.sgrfconferences.org/2016/NGBT/

http://www.nextgenerationsequencing-congress.com/

Announcements

6th International Conference on

Genomics & Pharmacogenomics

September 12-14, 2016 Berlin, Germany

http://genomics.conferenceseries.com/events-list/genome-engineering

OXFORD GLOBAL

6th Annual NGS Asia Congress 2016

11-12 October 2016, Singapore

http://www.ngsasia-congress.com/forthcoming-events/

ICBCSB 2017 : 19th International Conference on Bioinformatics and Computational Systems Biology

https://www.waset.org/conference/2017/01/sydney/ICBCSB

ANAKINRA

Anakinra, a recombinant interleukin-1 receptor antagonist (IL-1Ra) is utilized to remedy rheumatoid arthritis. Compared to normal humanoid IL-1Ra, anakinra has an extra methionine residue at its amino end. By competitively preventing the binding of IL-1 to the interleukin-1 type receptor, anakinra prevents the biologic activity of naturally occurring IL-1, responsible for inflammation and cartilage degradation responsible for rheumatoid arthritis. IL-1 is generated in answer to inflammatory signals and induces different physiologic reactions such as immunologic and inflammatory reactions.

Moreover, IL-1 causes tissue lesion like cartilage decadence and robust bone resorption. The natural IL-1 receptor antagonist exists in synovium and synovial fluid of the human body and counteracts the enhanced IL-1 concentrations. In patients with rheumatoid arthritis the natural IL-1 receptor antagonist is not detected in efficient concentrations. Anakinra is known as a "biological response modifier" since it is capable to target the pathologic factor of the disease. The non-glycosylated, recombinant anakinra is prepared from cultures of the bacterium *Escherichia coli* utilizing recombinant DNA technology. It contains of 153 amino acids in length and has a molecular weight of 17.3 kD. Anakinra is indicated to prevent the progression of structural injury associated with rheumatoid arthritis and for the management of symptoms of the disease in adults with moderately to severely active disease.

Reference: https://en.wikipedia.org/wiki/Anakinra

C1 ESTERASE SUPPRESSOR

C1 esterase suppressor is a protease suppressor and its key role is the prevention of the complement system to inhibit spontaneous activation. The failure of this protein is responsible for hereditary angioedema, or swelling due to leak of fluid from blood vessels into the extracellular matrix. Humans with repeated attacks of angioedema are usually treated applying androgens-based drugs including danazol and oxandrolone. Blood-derived C1-suppressor may be more efficient and does not have the side effects of androgens such as a risk of liver tumors and virilization. However, C1-suppressor may carry the risk related to the utilizing of any human blood product.

Cinryze is a pasteurized, highly purified and nanofiltered plasma-resultant C1 esterase suppressor. It was licensed for the utilization of HAE in 2008. Conestat alfa is a recombinant C1 suppressor obtained from the milk of transgenic rabbits. Ruconest is accepted for the cure of severe HAE shocks in adults.

Reference: https://en.wikipedia.org/wiki/C1-inhibitor

HEP G2 CELLS

Hep G2 is a cancer cell line obtained from the liver tissue of a particular man with a well-separated hepatic carcinoma. These cells have epithelial shape, and are not tumorigenic in murine. These cells are a favorable *in vitro* model for the investigation of diverged human hepatocytes. Owing to their high grade of functional and morphological differentiation *in vitro*, Hep G2 cells are a good model to analyze the dynamics and intracellular transferring of bile capillaries and sinusoidal membrane lipids and proteins in human hepatocytes *in vitro*. This is significant for the investigation of human liver diseases that are begun by an inaccurate subcellular transport of cell surface proteins, comprising Dubin-Johnson Syndrome, familial hypercholesterolemia and progressive familial intrahepatic cholestasis. Moreover, Hep G2 cells and their derivatives are applied as a model system for analysis of liver toxicity and metabolism of xenobiotics, the recognition of dietary and environmental genotoxic and cytotoxic agents, for drug targeting trainings and comprehending hepatocarcinogenesis. Besides, Hep G2 cells are utilized in trials with bio-artificial liver devices.

Reference: https://en.wikipedia.org/wiki/Hep_G2