Functional variants of human papillomavirus type 16 demonstrate host genome integration and transcriptional alterations corresponding to their unique cancer epidemiology.

*BMC genomics* : 851

**Summary**

Human papillomaviruses (HPVs) are a worldwide burden as they are a widespread group of tumour viruses in humans. Having a tropism for mucosal tissues, high-risk HPVs are detected in nearly all cervical cancers. HPV16 is the most common high-risk type but not all women infected with high-risk HPV develop a malignant tumour. Likely relevant, HPV genomes are polymorphic and some HPV16 single nucleotide polymorphisms (SNPs) are under evolutionary constraint instigating variable oncogenicity and immunogenicity in the infected host.

Combination of Carboplatin and Bevacizumab Is an Efficient Therapeutic Approach in Retinoblastoma Patient-Derived Xenografts.

*Investigative ophthalmology & visual science* : 4916-4926 : [DOI: 10.1167/iovs.15-18725]

**Summary**

Retinoblastoma (Rb) is a rare childhood cancer of the retina with a survival rate of 95% in children living in high-income countries, after appropriate therapies such as chemotherapy, local ophthalmologic treatment, and radiotherapy. However, due to inactivation of the RB1 gene, all bilateral and almost 15% of unilateral retinoblastoma patients have a higher risk of secondary cancers, especially sarcomas. Hence, new nonmutagen treatments are warranted. Therefore, we investigated the efficacy of therapy using anti-VEGF antibody bevacizumab, either alone or with carboplatin, in well-characterized Rb patient-derived xenografts (PDXs).

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Ligand-receptor dissociated expression explains high TSLP without prognostic impact in human primary head and neck squamous cell carcinoma.

Oncoimmunology : e1179414 : DOI : 10.1080/2162402X.2016.1179414

Summary

Thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7-like cytokine expressed by epithelial cells during allergic inflammation, and activating dendritic cells (DC). Its expression and functional role in cancer remain controversial. We conducted retrospective (n = 89), and prospective studies including patients with untreated primary head and neck squamous cell carcinoma (HNSCC). We found that TSLP was overexpressed by HNSCC tumor cells, and associated with a highly differentiated status. However, no significant difference in overall and recurrence-free survival was found between patients bearing a tumor with high and low TSLP levels, respectively. Surprisingly, there was no significant association between the levels of TSLP expression, and the number of tumor-infiltrating mature DCLAMP(+) DC. In order to explain the apparent lack of TSLP-induced DC activation, we performed phenotypic and functional experiments on freshly resected tumors. Tumor-infiltrating immune cells, including DC, did not express the TSLP receptor heterodimer (TSLPR chain, IL-7Ralpha chain). Furthermore, freshly sorted blood CD11c(+) DC from healthy donors cultured with tumor-conditioned supernatant exhibited an activated profile, but this was not affected by an anti-TSLP blocking antibody, suggesting a DC activation pathway independent of tumor-derived TSLP. Overall, our results demonstrate that TSLP is overexpressed in HNSCC but its function is hampered by the lack of TSLPR-expressing cells in the tumor microenvironment. Such a dissociated ligand-receptor expression may impact intercellular communication in other immune activation pathways, and tumor types.

Cristina Ghirelli, Benjamin Sadacca, Fabien Reyal, Raphaël Zollinger, Paula Michea, Philémon Sirven, Lucia Pattarini, Carolina Martínez-Cingolani, Maude Guillot-Delost, André Nicolas, Alix Scholer-Dahirel, Vassili Soumelis (2016 Sep 14)

No evidence for TSLP pathway activity in human breast cancer.

Oncoimmunology : e1178438 : DOI : 10.1080/2162402X.2016.1178438

Summary

Thymic stromal lymphopoietin (TSLP) is an epithelial cell-derived cytokine that primes dendritic cells for Th2 induction. It has been implicated in different types of allergic diseases. Recent work suggested that TSLP could play an important role in the tumor microenvironment and influence tumor progression, in particular in breast cancer. In this study we systematically assessed the production of TSLP at the mRNA and protein levels in several human breast cancer cell lines, large-scale public transcriptomics data sets, and primary human breast tumors. We found that TSLP production was marginal, and concerned less than 10% of the tumors, with very low mRNA and protein levels. In most cases TSLP was undetectable and found to be expressed at lower levels in breast cancer as compared to normal breast tissue. Last, we could not detect any functional TSLP receptor (TSLPR) expression neither on hematopoietic cells nor on stromal cells within the primary tumor.
microenvironment. We conclude that TSLP-TSLPR pathway activity is not significantly detected within human breast cancer. Taken together, these observations do not support TSLP targeting in breast cancer.


Mek1 Down Regulates Rad51 Activity during Yeast Meiosis by Phosphorylation of Hed1.

PLoS genetics : e1006226 : DOI : 10.1371/journal.pgen.1006226

Summary

During meiosis, programmed double strand breaks (DSBs) are repaired preferentially between homologs to generate crossovers that promote proper chromosome segregation at Meiosis I. In many organisms, there are two strand exchange proteins, Rad51 and the meiosis-specific Dmc1, required for interhomolog (IH) bias. This bias requires the presence, but not the strand exchange activity of Rad51, while Dmc1 is responsible for the bulk of meiotic recombination. How these activities are regulated is less well established. In dmc1Δ mutants, Rad51 is actively inhibited, thereby resulting in prophase arrest due to unrepaired DSBs triggering the meiotic recombination checkpoint. This inhibition is dependent upon the meiosis-specific kinase Mek1 and occurs through two different mechanisms that prevent complex formation with the Rad51 accessory factor Rad54: (i) phosphorylation of Rad54 by Mek1 and (ii) binding of Rad51 by the meiosis-specific protein Hed1. An open question has been why inhibition of Mek1 affects Hed1 repression of Rad51. This work shows that Hed1 is a direct substrate of Mek1. Phosphorylation of Hed1 at threonine 40 helps suppress Rad51 activity in dmc1Δ mutants by promoting Hed1 protein stability. Rad51-mediated recombination occurring in the absence of Hed1 phosphorylation results in a significant increase in non-exchange chromosomes despite wild-type levels of crossovers, confirming previous results indicating a defect in crossover assurance. We propose that Rad51 function in meiosis is regulated in part by the coordinated phosphorylation of Rad54 and Hed1 by Mek1.

Raphaëlle Laureau, Sophie Loeillet, Francisco Salinas, Anders Bergström, Patricia Legoix-Né, Gianni Liti, Alain Nicolas (2016 Feb 2)

Extensive Recombination of a Yeast Diploid Hybrid through Meiotic Reversion.

PLoS genetics : e1005781 : DOI : 10.1371/journal.pgen.1005781

Summary

In somatic cells, recombination between the homologous chromosomes followed by equational segregation leads to loss of heterozygosity events (LOH), allowing the expression of recessive alleles and the production of novel allele combinations that are potentially beneficial upon Darwinian selection. However, inter-homolog recombination in somatic cells
is rare, thus reducing potential genetic variation. Here, we explored the property of S. cerevisiae to enter the meiotic developmental program, induce meiotic Spo11-dependent double-strand breaks genome-wide and return to mitotic growth, a process known as Return To Growth (RTG). Whole genome sequencing of 36 RTG strains derived from the hybrid S288c/SK1 diploid strain demonstrates that the RTGs are bona fide diploids with mosaic recombined genome, derived from either parental origin. Individual RTG genome-wide genotypes are comprised of 5 to 87 homozygous regions due to the loss of heterozygous (LOH) events of various lengths, varying between a few nucleotides up to several hundred kilobases. Furthermore, we show that reiteration of the RTG process shows incremental increases of homozygosity. Phenotype/genotype analysis of the RTG strains for the auxotrophic and arsenate resistance traits validates the potential of this procedure of genome diversification to rapidly map complex traits loci (QTLs) in diploid strains without undergoing sexual reproduction.

Year of publication 2015

Etienne Muller, Baptiste Brault, Allyson Holmes, Angelina Legros, Emmanuelle Jeannot, Maura Campitelli, Antoine Rousselin, Nicolas Goardon, Thierry Frébourg, Sophie Krieger, Hubert Crouet, Alain Nicolas, Xavier Sastre, Dominique Vaur, Laurent Castéra (2015 Jul 10)

Genetic profiles of cervical tumors by high-throughput sequencing for personalized medical care.

Cancer medicine : 1484-93 : DOI : 10.1002/cam4.492

Summary

Cancer treatment is facing major evolution since the advent of targeted therapies. Building genetic profiles could predict sensitivity or resistance to these therapies and highlight disease-specific abnormalities, supporting personalized patient care. In the context of biomedical research and clinical diagnosis, our laboratory has developed an oncogenic panel comprised of 226 genes and a dedicated bioinformatic pipeline to explore somatic mutations in cervical carcinomas, using high-throughput sequencing. Twenty-nine tumors were sequenced for exons within 226 genes. The automated pipeline used includes a database and a filtration system dedicated to identifying mutations of interest and excluding false positive and germline mutations. One-hundred and seventy-six total mutational events were found among the 29 tumors. Our cervical tumor mutational landscape shows that most mutations are found in PIK3CA (E545K, E542K) and KRAS (G12D, G13D) and others in FBXW7 (R465C, R505G, R479Q). Mutations have also been found in ALK (V1149L, A1266T) and EGFR (T259M). These results showed that 48% of patients display at least one deleterious mutation in genes that have been already targeted by the Food and Drug Administration approved therapies. Considering deleterious mutations, 59% of patients could be eligible for clinical trials. Sequencing hundreds of genes in a clinical context has become feasible, in terms of time and cost. In the near future, such an analysis could be a part of a battery of examinations along the diagnosis and treatment of cancer, helping to detect sensitivity or resistance to targeted therapies and allow advancements towards personalized oncology.
G-quadruplexes (G4) are polymorphic four-stranded structures formed by certain G-rich nucleic acids, with various biological roles. However, structural features dictating their formation and/or function in vivo are unknown. In S. cerevisiae, the pathological persistency of G4 within the CEB1 minisatellite induces its rearrangement during leading-strand replication. We now show that several other G4-forming sequences remain stable. Extensive mutagenesis of the CEB25 minisatellite motif reveals that only variants with very short (≤ 4 nt) G4 loops preferentially containing pyrimidine bases trigger genomic instability. Parallel biophysical analyses demonstrate that shortening loop length does not change the monomorphic G4 structure of CEB25 variants but drastically increases its thermal stability, in correlation with the in vivo instability. Finally, bioinformatics analyses reveal that the threat for genomic stability posed by G4 bearing short pyrimidine loops is conserved in C. elegans and humans. This work provides a framework explanation for the heterogeneous instability behavior of G4-forming sequences in vivo, highlights the importance of structure thermal stability, and questions the prevailing assumption that G4 structures with short or longer loops are as likely to form in vivo.
Inactivating germline mutations in the tumour suppressor gene BRCA1 are associated with a significantly increased risk of developing breast and ovarian cancer. A large number (>1500) of unique BRCA1 variants have been identified in the population and can be classified as pathogenic, non-pathogenic or as variants of unknown significance (VUS). Many VUS are rare missense variants leading to single amino acid changes. Their impact on protein function cannot be directly inferred from sequence information, precluding assessment of their pathogenicity. Thus, functional assays are critical to assess the impact of these VUS on protein activity. BRCA1 is a multifunctional protein and different assays have been used to assess the impact of variants on different biochemical activities and biological processes.

Summary

CEB1 is a highly polymorphic human minisatellite. In yeast, the size variation of CEB1 tandem arrays has been associated with the capacity of the motif to form G-quadruplexes. Here we report on the NMR solution structure of a G-quadruplex formed by the CEB1 DNA G-rich fragment d(AGGGGGAGGGAGGGTGG), harboring several G-tracts including one with six continuous guanines. This sequence forms a dimeric G-quadruplex involving the stacking of two subunits, each being a unique snapback parallel-stranded scaffold with three G-tetrad layers, three double-chain-reversal loops, and a V-shaped loop. The two subunits are stacked at their 5'-end tetrad, and multiple stacking rotamers may be present due to a high symmetry at the stacking interface. There is a conformational exchange in the millisecond time scale involving a swapping motion between two bases of the six-guanine tract. Our results not only add to the understanding of how the G-quadruplex formation in human minisatellite leads to genetic instability but also address the fundamental questions.
regarding stacking of G-quadruplexes and how a long continuous G-tract participates in the structure and conformational dynamics of G-quadruplexes.

The FEN1 E359K germline mutation disrupts the FEN1-WRN interaction and FEN1 GEN activity, causing aneuploidy-associated cancers.

Oncogene : 902-11 : DOI : 10.1038/onc.2014.19

Summary

Polymorphisms and somatic mutations in Flap Endonuclease 1 (FEN1), an essential enzyme involved in DNA replication and repair, can lead to functional deficiencies of the FEN1 protein and a predisposition to cancer. We identified a FEN1 germline mutation that changed residue E359 to K in a patient whose family had a history of breast cancer. We determined that the E359K mutation, which is in the protein-protein domain of FEN1, abolished the interaction of FEN1 with Werner syndrome protein (WRN), an interaction that is critical for resolving stalled DNA replication forks. Furthermore, although the flap endonuclease activity of FEN1 E359K was unaffected, it failed to resolve bubble structures, which require the FEN1 gap-dependent endonuclease activity. To determine the etiological significance of E359K, we established a mouse model containing this mutation. E359K mouse embryonic fibroblasts (MEF) were more sensitive to DNA crosslinking agents that cause replication forks to stall. Cytological analysis suggested that the FEN1-WRN interaction was also required for telomere stability; mutant cell lines had fragile telomeres, increased numbers of spontaneous chromosomal anomalies and higher frequencies of transformation. Moreover, the incidence of cancer was significantly higher in mice homozygous for FEN1 E359K than in wild-type mice, suggesting that the FEN1 E359K mutation is oncogenic.

Claire Jubin, Alexandre Serero, Sophie Loeillet, Emmanuel Barillot, Alain Nicolas (2014 Feb 22)
Sequence profiling of the Saccharomyces cerevisiae genome permits deconvolution of unique and multialigned reads for variant detection.

G3 (Bethesda, Md.) : 707-15 : DOI : 10.1534/g3.113.009464

Summary

Advances in high-throughput sequencing (HTS) technologies have accelerated our knowledge of genomes in hundreds of organisms, but the presence of repetitions found in every genome raises challenges to unambiguously map short reads. In particular, short polymorphic reads that are multialigned hinder our capacity to detect mutations. Here, we present two complementary bioinformatics strategies to perform more robust analyses of genome content and sequencing data, validated by use of the Saccharomyces cerevisiae fully sequenced genome. First, we created an annotated HTS profile for the reference genome, based on the production of virtual HTS reads. Using variable read lengths and different numbers of mismatches, we found that 35 nt-reads, with a maximum of 6
mismatches, targets 89.5% of the genome to unique (U) regions. Longer reads consisting of 50-100 nt provided little additional benefits on the U regions extent. Second, to analyze the remaining multialigned (M) regions, we identified the intragenomic single-nucleotide variants and thus defined the unique (MU) and multialigned (MM) subregions, as exemplified for the polymorphic copies of the six flocculation genes and the 50 Ty retrotransposons. As a resource, the coordinates of the U and M regions of the yeast genome have been added to the Saccharomyces Genome Database (www.yeastgenome.org). The benefit of this advanced method of genome annotation was confirmed by our ability to identify acquired single nucleotide polymorphisms in the U and M regions of an experimentally sequenced variant wild-type yeast strain.

Alexandre Serero, Claire Jubin, Sophie Loeillet, Patricia Legoix-Né, Alain G Nicolas (2014 Jan 21)
Mutational landscape of yeast mutator strains.
Proceedings of the National Academy of Sciences of the United States of America : 1897-902 :
DOI : 10.1073/pnas.1314423111

Summary

The acquisition of mutations is relevant to every aspect of genetics, including cancer and evolution of species on Darwinian selection. Genome variations arise from rare stochastic imperfections of cellular metabolism and deficiencies in maintenance genes. Here, we established the genome-wide spectrum of mutations that accumulate in a WT and in nine Saccharomyces cerevisiae mutator strains deficient for distinct genome maintenance processes: pol32Δ and rad27Δ (replication), msh2Δ (mismatch repair), tsa1Δ (oxidative stress), mre11Δ (recombination), mec1Δ tel1Δ (DNA damage/S-phase checkpoints), pif1Δ (maintenance of mitochondrial genome and telomere length), cac1Δ cac3Δ (nucleosome deposition), and clb5Δ (cell cycle progression). This study reveals the diversity, complexity, and ultimate unique nature of each mutational spectrum, composed of punctual mutations, chromosomal structural variations, and/or aneuploidies. The mutations produced in clb5Δ/CCNB1, mec1Δ/ATR, tel1Δ/ATM, and rad27Δ/FEN1 strains extensively reshape the genome, following a trajectory dependent on previous events. It comprises the transmission of unstable genomes that lead to colony mosaics. This comprehensive analytical approach of mutator defects provides a model to understand how genome variations might accumulate during clonal evolution of somatic cell populations, including tumor cells.

Year of publication 2012

Laurent Acquaviva, Lóránt Székvölgyi, Bernhard Dichtl, Beatriz Solange Dichtl, Christophe de La Roche Saint André, Alain Nicolas, Vincent Géli (2012 Nov 15)
The COMPASS subunit Spp1 links histone methylation to initiation of meiotic recombination.
Science (New York, N.Y.) : 215-8 : DOI : 10.1126/science.1225739
Summary

During meiosis, combinatorial associations of genetic traits arise from homologous recombination between parental chromosomes. Histone H3 lysine 4 trimethylation marks meiotic recombination hotspots in yeast and mammals, but how this ubiquitous chromatin modification relates to the initiation of double-strand breaks (DSBs) dependent on Spo11 remains unknown. Here, we show that the tethering of a PHD-containing protein, Spp1 (a component of the COMPASS complex), to recombinationally cold regions is sufficient to induce DSB formation. Furthermore, we found that Spp1 physically interacts with Mer2, a key protein of the differentiated chromosomal axis required for DSB formation. Thus, by interacting with H3K4me3 and Mer2, Spp1 promotes recruitment of potential meiotic DSB sites to the chromosomal axis, allowing Spo11 cleavage at nearby nucleosome-depleted regions.