Année de publication : 1992

A Eychène, J V Barnier, F Apiou, B Dutrillaux, G Calothy (1992 Aug 1)
Chromosomal assignment of two human B-raf(Rmil) proto-oncogene loci: B-raf-1 encoding the p94Braf/Rmil and B-raf-2, a processed pseudogene.
Oncogene : 1657-60

Résumé

The B-raf gene is the human homolog of the avian c-Rmil proto-oncogene encoding a 94-kDa serine/threonine kinase detected in avian cells. We have previously shown that this protein contains amino-terminal sequences not found in other proteins of the mil/raf gene family. These sequences are encoded by three exons in the avian genome. We report that these three exons are conserved in the human B-raf gene and that they encode an amino acid sequence similar to that of the avian c-Rmil gene, indicating that in both avian and mammalian species the product of the B-raf/c-Rmil gene is a 94-kDa protein. We also identified two human B-raf loci: B-raf-1, located on chromosome 7q34, which encodes the functional B-raf/Rmil gene product, and B-raf-2, an inactive processed pseudogene located on chromosome Xq13.

A Eychène, J V Barnier, P Dézélée, M Marx, D Laugier, I Calogeraki, G Calothy (1992 Jul 1)
Quail neuroretina c-Rmil(B-raf) proto-oncogene cDNAs encode two proteins of 93.5 and 95 kDa resulting from alternative splicing.
Oncogene : 1315-23

Résumé

c-Rmil is the cellular allele of the v-Rmil oncogene transduced during in vitro passaging of Rous-associated virus type 1 in chicken embryonic neuroretina (NR) cells. The c-Rmil proto-oncogene is the avian homolog of the mammalian B-raf gene and belongs to the mil/raf oncogene family of serine/threonine protein kinases. The c-Rmil/B-raf gene is preferentially expressed in avian and mammalian neural tissues. Two c-Rmil cDNA species, resulting from an alternative splicing mechanism, were isolated from quail embryonic NR cDNA libraries. They encode two proteins of 767 and 807 amino acids that differ by the presence of an alternative exon, located upstream of the kinase domain. Expression of these cDNAs in COS-1 cells leads to the synthesis of two proteins with apparent molecular weights of 93.5 and 95 kDa, recognized by an Rmil-specific antiserum. Both proteins are phosphorylated in an immune complex kinase assay. A protein of 94 kDa is also immunoprecipitated in avian NR cells and is identical to the 93.5-kDa protein expressed in COS-1 cells, as shown by Staphylococcus aureus V8 protease mapping. The c-Rmil proteins contain the three conserved regions previously identified in mil/raf protein kinases. In addition, they contain amino-terminal sequences that are not present in the other mil/raf proteins identified to date. These additional sequences may define a novel functional domain for c-Rmil/B-raf and could play a role in signal transduction in neural cells.

Année de publication : 1991

M P Felder, A Eychène, J V Barnier, I Calogeraki, G Calothy, M Marx (1991 Jul 1)
Common mechanism of retrovirus activation and transduction of c-mil and c-Rmil in chicken neuroretina cells infected with Rous-associated virus type 1.
Journal of virology : 3633-40

Résumé

We previously described the isolation of the IC10 retrovirus which transduced the v-Rmil oncogene, a new member of the mil/raf gene family. This virus was generated during serial passaging of Rous-associated virus type 1 (RAV-1) in chicken embryo neuroretina (NR) cells and was selected for its ability to induce proliferation of these nondividing cells. IC10 was isolated after six passages of culture supernatants but was not detected in proliferating NR cells during early virus passages. In this study, we molecularly cloned and sequenced another v-Rmil-containing provirus, designated IC11, from NR cells infected at the third virus passage of the same experiment. Both IC11 and IC10 transduced only the serine/threonine kinase domain of c-Rmil. Comparison of v-Rmil and c-Rmil sequences indicated that amino-terminal truncation is sufficient to activate the mitogenic properties of c-Rmil. IC11 and IC10 have identical 3′ ends but differ by their 5′ RAV-1-Rmil junctions. The 3′ ends of both viruses were generated by recombination between Rmil and env genes, involving partial sequence identity. The 5′ RAV-1-Rmil junction of IC11 was formed by a splicing process between the RAV-1 leader and a 37-bp c-Rmil exon located upstream of the kinase domain. NR cells infected with this virus synthesize a unique Rmil protein. IC10 contains most of the gag gene recombined with v-Rmil and encodes a gag-Rmil hybrid protein. Serial passaging of IC11 in NR cells led to the formation of a gag-Rmil-containing retrovirus. These results indicate that IC11 represents an early step in transduction and that this virus further recombined with RAV-1 to generate IC10. They confirm our previously proposed model for the multistep generation of v-mil-transducing retroviruses. Therefore, activation and transduction of c-mil and c-Rmil, in NR cells infected with RAV-1, result from a common mechanism.

Année de publication : 1990

A Eychène, C Béchade, M Marx, D Laugier, P Dezélée, G Calothy (1990 Jan 1)
Molecular and biological properties of c-mil transducing retroviruses generated during passage of Rous-associated virus type 1 in chicken neuroretina cells.
Journal of virology : 231-8

Résumé

IC1, IC2, and IC3 are novel c-mil transducing retroviruses generated during serial passaging of Rous-associated virus type 1 (RAV-1) in chicken embryo neuroretina cells. They were isolated by their ability to induce proliferation of these nondividing cells. IC2 and IC3 were generated during early passages of RAV-1 in neuroretina cells, whereas IC1 was isolated after six consecutive passages of virus supernatants. We sequenced the transduced genes and the mil-RAV-1 junctions of the three viruses. The 5′ RAV-1-mil junction of IC2 and IC3 was

formed by a splicing process between the RAV-1 leader sequence and exon 8 of the c-mil gene. The 5′ end of IC1 resulted from homologous recombination between gag and mil sequences. Reconstitution experiments showed that serial passaging of IC2 in neuroretina cells also led to the formation of a gag-mil-containing retrovirus. Therefore, constitution of a U5-leader-delta c-mil-delta RAV-1-U3 virus represents early steps in c-mil transduction by RAV-1. This virus further recombined with RAV-1 to generate a gag-mil-containing virus. The three IC viruses transduced the serine/threonine kinase domain of the cellular gene. Hence, amino-terminal truncation is sufficient to activate the mitogenic property of c-mil. Comparison of the transforming properties of IC2 and IC1 showed that the transduced mil gene, expressed as a unique protein independent of gag sequences, was weakly transforming in avian cells. Acquisition of gag sequences by IC1 not only increased the rate of virus replication but also enhanced the transforming capacity of the virus.

Année de publication : 1989

A Eychène, M Marx, P Dezélée, G Calothy (1989 Feb 11)

Complete nucleotide sequence of IC10, a retrovirus containing the Rmil oncogene transduced in chicken neuroretina cells infected with avian retrovirus RAV-1.

Nucleic acids research : 1250

Résumé

Année de publication : 1988

M Marx, P Crisanti, A Eychène, C Béchade, D Laugier, J Ghysdaël, B Pessac, G Calothy (1988 Dec 1)

Activation and transduction of c-mil sequences in chicken neuroretina cells induced to proliferate by infection with avian lymphomatosis virus.

Journal of virology : 4627-33

Résumé

We report that nondividing neuroretina cells from chicken embryos can be induced to proliferate following infection with Rous-associated virus type 1 (RAV-1), an avian lymphomatosis retrovirus lacking transforming genes. Multiplication of RAV-1-infected neuroretina cells is observed after a long latency period and takes place initially in a small number of cells. We also show that serial virus passaging onto fresh neuroretina cultures leads to the generation of novel mitogenic viruses containing the mil oncogene. DNA analysis indicated that RAV-1 is the only provirus detected in cells infected at virus passage 1, whereas neuroretina cells infected at subsequent virus passages harbor mil-containing proviruses. Three viruses, designated IC1, IC2, and IC3, were molecularly cloned. Restriction mapping indicated that in each virus, truncated c-mil sequences were inserted within different portions of the RAV-1 genome. In addition, IC1 and IC2 viruses have transduced

novel sequences that belong to the 3′ noncoding portion of the c-mil locus. All three viruses induce neuroretina cell multiplication and direct the synthesis of mil-specific proteins. Proliferation of neuroretina cells infected at passage 1 of RAV-1 was not associated with any detectable rearrangement of c-mil, when a v-mil probe was used. However, these cells expressed high levels of an aberrant 2.8-kilobase mRNA hybridizing to mil but not to a long terminal repeat probe. Therefore, transcriptional activation of a portion of c-mil could represent the initial events induced by RAV-1 infection and lead to retroviral transduction of activated c-mil sequences.

M Marx, A Eychène, D Laugier, C Béchade, P Crisanti, P Dezélée, B Pessac, G Calothy (1988 Nov 1)

A novel oncogene related to c-mil is transduced in chicken neuroretina cells induced to proliferate by infection with an avian lymphomatosis virus.

The EMBO journal : 3369-73

Résumé

Non-dividing neuroretina cells from chicken embryos are induced to proliferate after a long latency, following infection with Rous associated virus type 1, an avian retrovirus which does not carry a transforming gene. We have isolated from these proliferating cells an acutely mitogenic retrovirus, designated IC10, which contains a novel oncogene. Nucleotide sequencing showed that the IC10 virus has transduced 1101 nucleotides of cellular origin inserted between the gag and env genes of RAV-1. This oncogene, designated v-Rmil, is 70.1% homologous to v-mil. v-Rmil encodes a protein of 40,976 daltons sharing 83.8% homology with the catalytic domain of the v-mil protein. Divergence with the v-mil gene product is observed at the NH2- and COOH-terminal portions of the v-Rmil protein. Restriction analysis of normal chicken DNA indicated that v-Rmil is derived from a cellular gene distinct from c-mil. The c-Rmil gene is transcribed through a major mRNA, greater than 10 kb in length, that is detected at much higher levels in neuroretinas, as compared to other embryonic tissues.