

Solid Tumour Section

Short Communication

inv(X)(p11.4p11.22) BCOR/CCNB3 in bone sarcoma

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Identity

Other names

BCOR-CCNB3 positive bone sarcoma
BCOR-CCNB3 Ewing-like tumor

Note

BCOR-CCNB3 Ewing-like tumors form a histologically heterogeneous family belonging to the group of small round cell tumors, just as EWS-ETS positive "classical" Ewing.

Clinics and pathology

Disease

Bone sarcoma

Note

Description of this new clinical entity in the literature is recent (Pierron et al., 2012). Clinical data of this small cohort of patients is under current study and only few pieces can be displayed for now.

Epidemiology

BCOR-CCNB3 RT-PCR screening of a series of transcript-negative sarcomas yielded a total of 24 positive cases, out of 594 (4%), meaning this entity is rare among the rare transcript-negative sarcomas.

Clinics

Clinical description is highly similar to that of

Ewing sarcoma, with a median age of occurrence of 13 years, a sex ratio disequilibrium towards males, and tumors affecting preferentially long bones, the spine and pelvis. 20% of patients presented with a soft tissue tumor.

Pathology

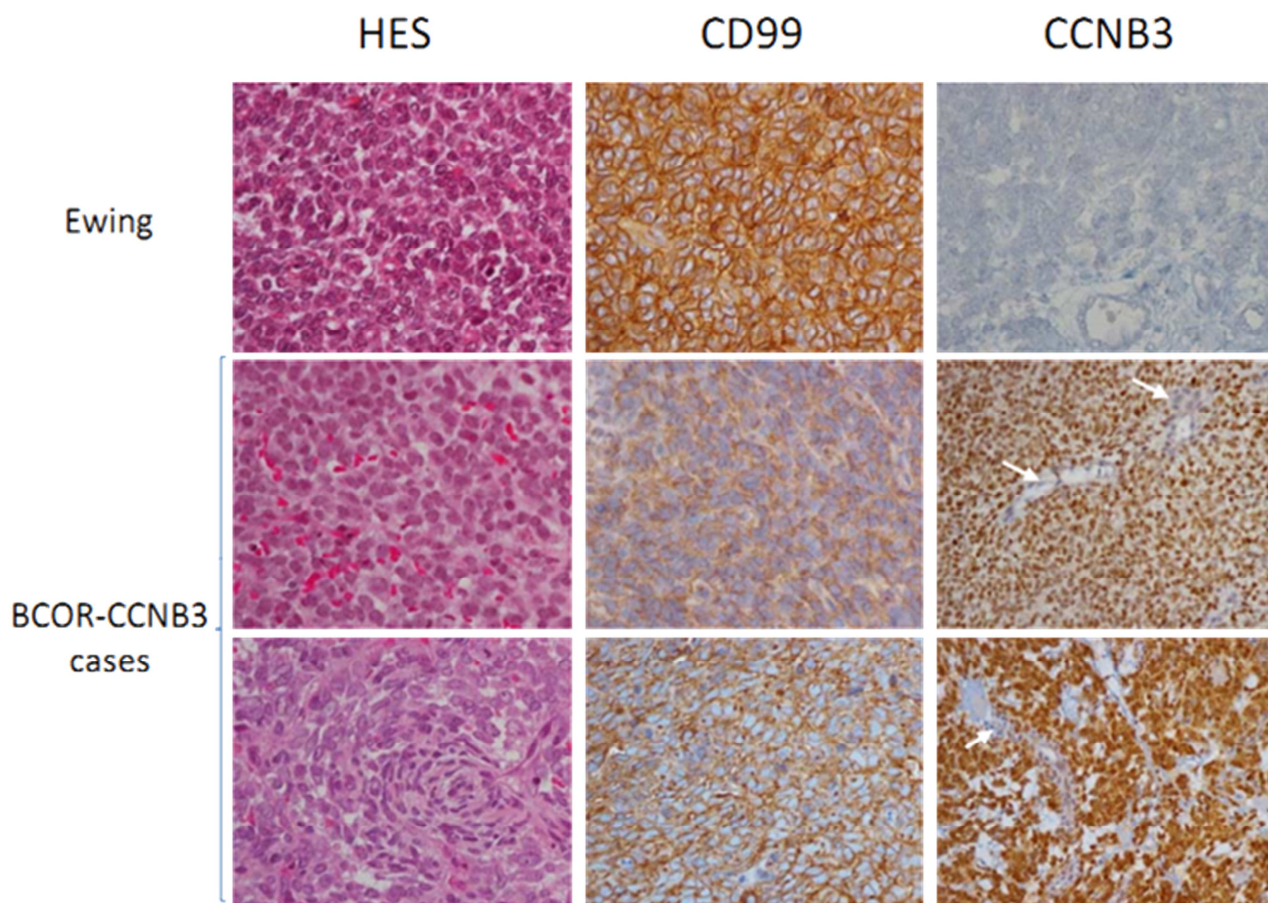
Pathological reports were consistent with an undifferentiated, small round cell sarcoma, suggestive of the Ewing sarcoma family of tumors. However, in contrast to Ewing sarcoma, approximately half of the cases lacked strong membrane positivity for CD99. BCOR-CCNB3-positive cases that were tested exhibited strong nuclear CCNB3 staining. This finding highlights the potential usefulness of a simple CCNB3 immunohistochemical assay as a diagnostic test for this subgroup of sarcoma.

Treatment

Due to the recent description of this entity, retrospective work on received treatment is on-going. Still, the treatment of these tumors is generally based on combined therapy with chemotherapy associated to best local treatment.

Prognosis

Retrospective work on outcome of patients harboring BCOR-CCNB3 positive tumors is on-going. As in all sarcomas, prognosis might be influenced by the presence of metastases at the time of diagnosis.



Hematoxylin and eosin staining and immunohistochemical analysis of CD99 and CCNB3 in a Ewing case as well as in two BCOR-CCNB3-positive cases. Courtesy Jean-Michel Coindre.

Cytogenetics

Cytogenetics Morphological

BCOR-CCNB3 Ewing-like tumors show a X-chromosome paracentric inversion; the inversion results in fusion of the complete coding sequence of the BCOR gene to the last 8 exons of CCNB3, leading to a hybrid transcript and an oncogenic chimeric protein.

Additional anomalies

SNP6.0 arrays detected no alteration in 11 of the 18 BCOR-CCNB3 Ewing-like cases studied. In the other seven cases, the only recurrent abnormalities were deletions at chromosomes 17p and 10q, each observed in two cases.

Neither deletion nor amplification of the fusion partners was observed.

Of note, the most frequent copy-number abnormalities of Ewing sarcoma, particularly the gain at chromosome 8 that is observed in ~50% of Ewing sarcoma cases, and the gain of 1q or chromosome 12 or the loss of 16q, which are each

observed in ~25% of Ewing sarcoma cases, were not recurrent characteristics of BCOR-CCNB3-positive cases.

These data indicate that classic Ewing sarcomas and BCOR-CCNB3-positive tumors do not share common copy-number changes.

Genes involved and proteins

BCOR (BCL6 corepressor)

Location

Xp11.4

Protein

Interacting co-repressor of BCL6.

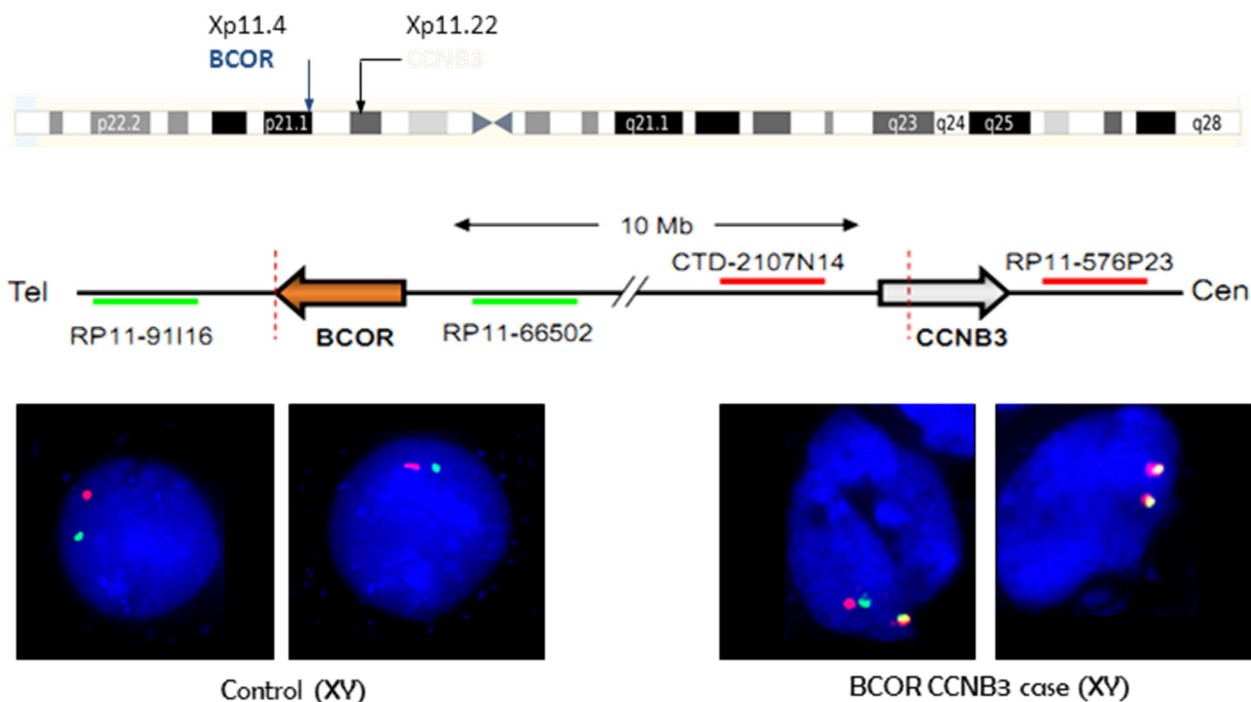
CCNB3 (cyclin B3)

Location

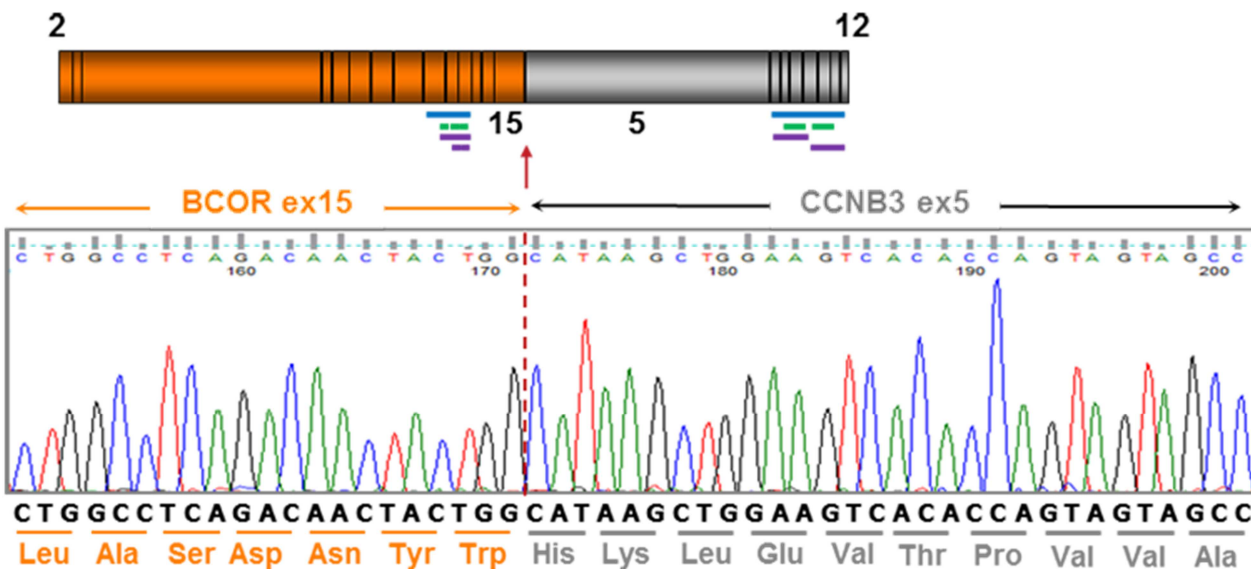
Xp11.22

Protein

Cyclin B3, early meiotic cyclin in spermatogenesis.



A paracentric inversion on the X chromosome was seen in FISH experiments. BACs flanking BCOR were labeled with FITC, and BACs flanking CCNB3 were labeled with rhodamine. In control cells, (a lymphoblastoid cell line), the two red spots (or the two green spots) are in close proximity and appear either as a single spot or as unicolor doublets. In BCOR-CCNB3-positive cells with paracentric inversion on the X chromosome, the two red and green spots are split and appear as bicolored doublets.



Structure of the BCOR-CCNB3 cDNA. The sequence of the BCOR-CCNB3 junction is shown, with the encoded protein sequence reported below it. Red dashed line and arrow indicate the fusion point.

Result of the chromosomal anomaly

Hybrid Gene

Transcript

The complete coding sequence of the BCOR gene is fused to the last 8 exons of CCNB3, leading to a hybrid

transcript and an oncogenic chimeric protein. The stop codon of BCOR (TGA) is included within a putative GGTGAG donor splice-site sequence. Thus, the most likely hypothesis accounting for the fusion mRNA is that this cryptic donor site is spliced with the acceptor site of exon 5 of CCNB3. Splicing was found to be incomplete, leading to both BCOR-CCNB3 and wild-type BCOR expression.

Detection

Tumors are snap frozen in liquid nitrogen. After crushing tumors, we isolate total RNA using the TRIzol reagent kit (Invitrogen). RNA is quantified with Qubit (Invitrogen) and Nanodrop ND1000 (ThermoFisher Scientific) before quality assessment with the Agilent 2100 Bioanalyzer. Total RNAs are randomly reverse transcribed (Random Hexamers, Applied Biosystems, FosterCity, CA) with GeneAmp RNA PCR Core Kit (Applied Biosystems, FosterCity, CA) in three steps (75°C - 5'; 42°C - 30'; 85°C - 5') on a Thermomixer Comfort (Eppendorf, Hamburg, Germany).

100ng of tumoral cDNA are amplified by Taq Gold with buffer II (Applied Biosystems, FosterCity, CA) with BCOR3.1.

(5'-GGCAGGTTTCTGCAAGTCTC) and CCNB3.1.

(5'-AGATGCCTCCTCAGTGTGG) primers and sequenced by the same oligos.

Sequence should yield a unique and tumor specific PCR product of 290pb.

Fusion Protein**Description**

The full fusion protein is expected to have a molecular weight of 337 kDa and to include the three known ankyrin domains of BCOR as well as

the two known cyclin domains of CCNB3.

Oncogenesis

5-bromodeoxyuridine (BrdU) staining of mouse NIH3T3 fibroblasts overexpressing BCOR-CCNB3 or ΔCCNB3, a truncated version of CCNB3 corresponding to the portion involved in the fusion gene, was performed. Compared to the empty vector, both expression vectors led to a twofold increase in the proportion of cells in S phase. Although cell cycle analyses clearly show that the CCNB3 portion of the fusion is potentially sufficient to mediate cell cycle effects, it is expected that oncogenesis also relies on additional phenotypic effects from the full-length fusion protein.

References

Pierron G, Tirode F, Lucchesi C, Reynaud S, Ballet S, Cohen-Gogo S, Perrin V, Coindre JM, Delattre O. A new subtype of bone sarcoma defined by BCOR-CCNB3 gene fusion. *Nat Genet.* 2012 Mar 4;44(4):461-6

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