Autocrine IGF-1/IGF-1R signaling is responsible for constitutive PI3K/Akt activation in acute myeloid leukemia: therapeutic value of neutralizing anti-IGF-1R antibody

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Online Supplementary Table S1. Primers used to amplify IGF-1R cDNA or exons 15 and 16 of IGF-1R genomic DNA.

		PCR product size	Reaction mix specificity	Annealing T°
Primers used to amplify the complete cDNA (GenBank accession number X04434)				
R-1F	5'-GGAATTTCATCCCAAATAAAAGG-3'	573 bp	P	58°C
R-1R	5'-ACAGGTCCCCACATTCCTTT-3'			
R-2F	5'-GGATGCGGTGTCCAATAACT-3'	695 bp	S	60°C
R-2R	5'-AATGGCGGATCTTCACGTAG-3'			
R-3F	5'-AGGATGCACCATCTTCAAGG-3'	693 bp	P	60°C
R-3R	5'-TTGACGTAAACGGCGTACTG-3'			
R-4F	5'-GAAGCCCTGGACTCAGTACG-3'	699 bp	S	60°C
R-4R	5'-TGGATATCGATGCGGTACAA-3			
R-5F	5'-CGGAAGAGCTGGAGACAGAG-3'	713 bp	P	60°C
R-5R	5'-AAGTTCCCGGCTCATGGT-3'			
R-6F	5'-TCCATCTGATCATCGCTCTG-3'	569 bp	S	60°C
R-6R	5'-CATGCCGTCTGCAATCTCT-3'			
R-7F	5'-CCAACACTGGTCATCATGGA-3'	580 bp	P	60°C
R-7R	5'-TGATGCTGCTGATGATCTCC -3'			
R-8F	5'-GCATGGCATACCTCAACGC-3'	745 bp	S	60°C
R-8R	5'-AAGGATCAGCAGGTCGAAGA-3'			
Primers used to amplify exon 15 and 16 of genomic DNA (GenBank accession number NC_00015)				
D-15F	5'-CGCTAATCTCAGGCTACTGA-3'	373 bp	S	61°C
D-15R	5'-GCAACCCTCCTGAAAGCTC-3'			
D-16F	5'-CTGTGGGTTTAAGGAAGCAGC-3'	460 bp	S	61°C
D-16R	5'-CAGAAGGCAAAGGCAAGACA-3'			

Polymerase chain reactions (PCR) were performed in a 50 μL reaction mixture containing 100 ng of DNA or cDNA corresponding to 100 ng RNA equivalent, 0.2 mM dNTP, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$ or 2 mM $MgSO_4$, 10 pmol of forward and reverse primers and 1 unit of Taq DNA Polymerase Platinum (P) or Standard (S) (Invitrogen, France). Samples were initially denaturated at 92°C for 4 min and DNA amplification was achieved by 35 cycles of denaturating (94°C for 30 s), annealing (58 to 61°C for 40 s) and extension (72°C for 60 s) on an ABI 9700 PCR system (Applied Biosystems, France). Purified PCR products were directly sequenced with 3.3 pmol of each forward and reverse primer using the Big Dye Terminator Cycle sequencing kit (Applied Biosystems). Sequence analysis was performed on an ABI3130 instrument and mutations were determined using SeqScape software (Applied Biosystems).