references, patents and published patent applications cited throughout this application are expressly incorporated herein by reference.

Examples

Example 1: Generation and expression of Notch1 immunogen

Generation of Notch1 immunogen expression constructs

The immunogen constructs were generated by multiplexing PCR (Fig. 1) for monoclonal antibody (mAb) generation. As illustrated in Fig. 1, the Notch1 immunogen cDNA was synthesized by multiple overlapping PCR using the Notch1 full-length cDNA clone as template (OriGene, Cat. No. TC308883, Rockville, MD) and High Fidelity PCR reagent system following the manufacturer's protocol (Roche, Indianapolis, IN). The recombinant Notch1 immunogen cDNA, containing N-terminal leader peptide, EGF-like repeats 35-36, NRR, (including Lin A, B and C domains and the HD domain) and a small portion of intracellular sequence, was cloned in a Fc-fusion protein vector with Notch1 immunogen fused to the N-terminus of Fc sequence. The Notch1 immunogen plasmid, referred to as N1-NRR-TM(-), contains the cDNA insert shown in sequence 1 ~~in Figure 11,~~ (SEQ ID NO:1), which encodes the immunogen protein shown in sequence 2 ~~in Figure 11~~ (SEQ ID NO:2).

A similar plasmid was constructed in parallel as shown in Fig. 2, which contains the same sequence as N1-NRR-TM(-) except that the transmembrane (TM) sequence (the last 24 amino acid residues in Sequence 4) was used to replace the intracellular sequence (the last 44 amino acid residues in Sequence 2) of N1-NRR-TM(-). This PCR-amplified Notch1 immunogen cDNA was cloned in pcDNA3.1D/V5-His (Invitrogen). The plasmid was referred to as N1-NRR-TM(+). The nucleic acid sequence and amino acid sequence of N1-NRR-TM(+) is shown as Sequence 3 (SEQ ID NO:3) and 4 (SEQ ID NO:4) ~~in Figure 12~~.

Expression and purification of Notch1 immunogen protein

N1-NRR-TM(-) was expressed in Freestyle™ 293-F cells (Invitrogen, Inc., Calsbad, CA) by transient transfection using Freestyle™ Max Reagent (Invitrogen) and the manufacturer's protocol, verified by Western blot analysis. Briefly, 1 X 10^7 cells were seeded in a tissue culture shaker flask containing 30 milliliters (ml) of 293-F cell growth medium (Invitrogen). The secreted protein was analyzed by taking an aliquot of 0.5 ml

the tumor samples harvested from mice treated with N248A had a robust NICD reduction, which persisted until five days post dosing (Figure 10). In contrast, the control mAb, D16A, did not reduce NICD level (data not shown). The maximal inhibition of NICD by N248A at 5 mg/kg was approximately 80%.

Example 8: Cloning and sequences of Notch1 mAb, N248A

The sequences of the variable regions of mAb N248A was determined. The antibody IgG subtype was first assessed using an Isostrip Mouse Monoclonal Antibody kit (Roche Diagnostics, Indianapolis, IN). The results indicated that N248A has an IgG_1 heavy chain and a lambda light chain. For cloning and sequencing of mAb N248A, 1 x 10^6 hybridoma cells were harvested and lysed to isolate total cellular RNA using RNeasy Mini Reagent kit and manufacturer's protocol (Qiagen, Valencia, CA). The first strand cDNA was synthesized on the RNA templates using Superscript III reverse transcriptase (InVitrogen). The cDNAs of the variable regions of light chain and heavy chain were amplified by PCR from the first strand cDNA using degenerate forward primers complimentary to the 5'-end of mouse lambda chain coding sequence and a reverse primer matching the constant region adjacent to the 3'-end of the variable region, or using degenerate forward primers complementary to the 5'-end of mouse IgG1 heavy chain coding sequence and a respective IgG1 constant region reverse primer. PCR cycling conditions were as follows: 1 cycle at 96 C for 1 minute, followed by 40 cycles at 95 ˚C for 20 sec, 50 ˚C for 20 sec, and 72 ˚C for 30 second. The resulting PCR products were cloned into pCR-4-TOPO vector (Invitrogen), sequenced by conventional methods, and analyzed using Vector NTI Advance software, (InVitrogen). The cloned antibody sequences were confirmed by direct comparison with the N-terminal sequences obtained from purified hybridoma-derived antibody, as determined by Mass spectrometry (Univ. of CA, Davis, Molecular Structure Facility). The compiled sequence results demonstrated that the variable region of mAb N248A heavy chain contains 121 amino acid residues, and the light chain contains 109 amino acid residues. Further analysis of the N248A mAb V_H sequence and V_L sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 and light change CDR1, CDR2 and CDR3. The nucleotide and amino acid sequences of the heavy chain variable region CDR1, CDR2 and CDR3 of mAb N248A are shown ~~in Figure 15~~ as sequences 15-20 (SEQ ID NO: 15-20). The

nucleotide and amino acid sequences of the light chain variable region CDR1, CDR2 and CDR3 of mAb N248A are shown ~~in Figure 14~~ as sequences 9-14 (SEQ ID NO: 9-14). The nucleotide and amino acid sequences of the heavy chain variable region and light chain variable region of mAb N248A are shown ~~in Figure 13~~ as sequences 5-8 (SEQ ID NO: 5-8).