Supplementary Material (ESI) for Molecular BioSystems

Synthesis and Evaluation of Xylopyranoside Derivatives as "Decoy Acceptor" of Human β-1,4-GalT 7

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Supplementary Information

Heterologous expression and purification of a soluble form of human β-1,4-GalT 7.

DNA manipulation was according to standard procedures.[1] The coding sequence corresponding to the human β-1,4-GalT 7 was obtained by double digestion of the plasmid IRAUp969H0626D with *Eco*RI and *Xho*I. After digestion, two fragments of 7.0 and 7.1 kb respectively were obtained. The first fragment (named EcDNA) included the cDNA sequence of the human β-1,4-GalT 7 meanwhile the second fragment (named Ep969) contained the chloramphenicol resistance gene. A mixture of these fragments was ligated into the doubled digested vector pET-28b(+). The ligation mixture was transformed into *E. coli* DH5α competent cells. Recombinant plasmids harbouring the β-1,4-GalT 7 cDNA sequence (pEcDNA) were Kan^R but sensible to choramphenicol. After selection, a colony containing the plasmid pEcDNA was cultured in Luria-Bertani (LB) broth containing kanamycin (26 μg/mL) at 37 ºC with shaking. The plasmid was purified and sequenced to check the presence of the β-1,4-GalT 7 cDNA.

To remove the transmembrane fragment, after digestion with *Nhe*I and *Pst*I the plasmid pEcDNA was blunt-ended by removing the 3'OH overhang and filling-in the 3'OH recessed end with T4 DNA polymerase. To remove the 3'OH overhang end, the plasmid was incubated at room temperature with T4 DNA polymerase and dTTP (90 μM) in a total volume of 55 μL. After 5 min, the reaction mixture was supplemented with 90 μM dNTPs to fill-in the 3'OH recessed ends. After another 5 min, the reaction was stopped by heating to 70 ºC for 10 min. The plasmid was purified and ligated in a reaction mixture (50 mL) containing T4 DNA ligase and PEG 5000.[2] The resulting plasmid pE*b4g7* was transformed into *E. coli* BL21(DE3) competent cells.

A colony containing the plasmid pE*b4g7* was cultured in LB broth containing kanamycin (26 μg/mL) at 37 ºC with shaking. When the culture reached an $O.D_{600nm}$ of 0.5-0.6, β-1,4-GalT 7 expression was induced with IPTG (1.0 mM) and the temperature was dropped to 30 ºC. The culture was maintained over night (O/N) and after that, was centrifuged at 10,000 x g during 30 min at 4 ºC. The cells were disrupted in a French Press (1,100 psi) and the cell free extract (CFE) thus obtained was treated with DNase and streptomycin sulphate as described by Bastida *et al*.[3]

The recombinant protein containing an N-terminal 6xHis tag was purified by Immobilized Metal Affinity Chromatography (IMAC) using a Ni^{+2}-IDA-agarose column pre-equilibrated with Tris-HCl buffer (50 mM, pH 8.0). Ater loading the CFE, the column was washed with the same buffer containing imidazole (10 mM). Finally, the recombinant protein was eluted with the same buffer containing imidazole 0.5 M. All the fractions containing protein were pooled together and dialysed to remove the imidazole. SDS/PAGE analysis of the purified recombinant enzyme (Fig. S1), showed a homogeneous protein band matching the expected molecular weight for the β-1,4-GalT 7 soluble domain plus the His tag (34.3 kDa).

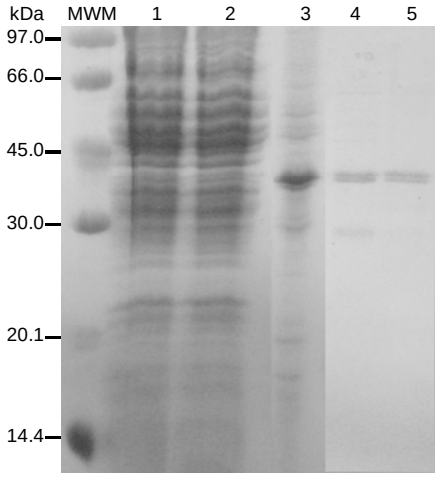

Fig.S1 SDS/PAGE analysis of the recombinant His-tagged β-1,4-GalT 7 soluble domain purification. MWM: Molecular weight markers; lanes 1 and 2: cell free extract; lane 3: recombinant β-1,4-GalT 7 bound to the IMAC resin; lanes 4 and 5: first and second fractions eluted from the IMAC column with imidazole. The image is a composition of two different gels.

Table S1. Effect of xylopyranoside derivatives **4**, **7**, **8**, **9**, **13** ,**14** and peracetate **24** and **25** on the proliferation of human lung carcinoma cells A549. (Mean±SD)

	% cell proliferation (mean±SD)							
μM	**4**	**7**	**8**	**9**	**13**	**14**	**24**	**25**
19	100±10	100±9	100±9	100±10	100±11	100±11	100±9	100±10
29	100±3	94±8	79±7	95±9	95±9	104±9	83±7	97±9
43	99±4	106±10	69±6	107±10	90±8	87±8	73±6	100±9
65	100±6	109±10	55±12	85±8	66±6	70±6	31±13	100±9
98	98±9	108±10	3±11	66±6	35±6	67±6	1±10	101±9
147	101±13	99±9	-60±5	13±14	-67±6	50±8	-91±8	96±9
220	90±16	100±9	-109±10	-33±3	-98±9	5±8	-147±13	100±8
330	100±10	97±9	-265±24	-181±16	-268±24	-60±5	-420±38	79±9

References.

1 J. Sambrook, E. F. Fritsch, T. Maniatis, in *Molecular cloning. A laboratory manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y, 2nd edn., 1989.
2 B. H. Pfeiffer, S. B. Zimmerman, *Nucleic Acids Res.* 1983, **11**, 7853-7871.
3 A. Bastida, A. Fernández-Mayoralas, R. G. Arrayás, F. Iradier, J. C. Carretero, E. García-Junceda, *Chem. Eur. J.* 2001, **7**, 2390-2397.