Influence of glycosylation on the structure of human natural killer cell receptor NKp30 in complex with its tumor ligand B7-H6

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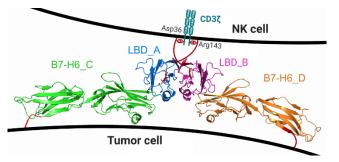
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NKp30 is an activating receptor on the surface of human natural killer (NK) cells. Its crystal structure has been published previously by Joyce *et al.* [1], PDB code 3NOI. B7-H6 is an activating immunoligand expressed by some tumor cells. Its structure in complex with NKp30 has been described by Li *et al.* [2], PDB code 3PV6.

In this study, we present a new crystal structure of NKp30:B7-H6 at resolution 3.1 Å using homogenously glycosylated proteins produced in HEK293S GnTI⁻ cell lines. The structure has been deposited in the Protein Data Bank under code 6YJP and published [3].

For the structural study, NKp30 was used with complete glycosylation, while B7-H6 was deglycosylated after the first GlcNAc for better crystallization. The new structure showed the same NKp30:B7-H6 interaction interface as observed by Li *et al.* (3PV6). Similarly, as in the structure of Joyce *et al.* (3NOI), NKp30 form dimers. However, the dimers of glycosylated NKp30 are different (the glycan presence hinders the formation of the dimers observed in PDB 3NOI), and according to Pisa server validation, the new dimers are more likely biologically relevant.

Furthermore, the asymmetric contains a dimer of NKp30 (contacts of chains A-C and B-paper [3] shows a hypothesis B7-H6 ligands during contact



unit of the new crystal structure placed among two B7-H6 molecules D_{symm}). The illustration taken from our of NKp30 dimer bound between two of the NK cell and the tumor cell.

- [1] Joyce, M.G., Tran, P., Zhuravleva, M.A., Jaw, J., Colonna, M., Sun, P.D. (2011) Proc. Natl. Acad. Sci. USA 108, 6223–6228.
- [2] Li, Y., Wang, Q., Mariuzza, R.A. (2011). J. Exp. Med. 208, 703-714.
- [3] Skořepa, O., Pazicky, S., Kalousková, B., Bláha, J., Abreu, C., Ječmen, T., Rosůlek, M., Fish, A., Sedivy, A., Harlos, K., Dohnálek, J., Skálová, T., Vaněk, O. (2020). Cancers 12, 1998.

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