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Dermatan sulphate is an activating ligand of anaplastic lymphoma kinase

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Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase (RTK) that harbours a tyrosine kinase domain in its intracellular region and is expressed in both central and peripheral nervous systems. RTKs are activated upon ligand binding and receptor clustering; however, ALK remains an orphan receptor despite its pathological significance, especially in malignancy. Recent biochemical work showed that heparan sulphate (HS), an unbranched sulphated glycan, acts as a ligand for and activates ALK. Here, we show that dermatan sulphate (DS, chondroitin sulphate B) directly interacts with the extracellular N-terminal region of ALK as well as HS. The tetrasaccharide of DS was required and was sufficient for inducing autophosphorylation of ALK at tyrosine 1604, a marker for activated ALK. Interestingly, longer oligosaccharides caused enhanced activation of ALK, as was the case for HS. Our results provide a novel example of glycans as signalling molecules and shed light on the pathophysiological roles of ALK.

Keywords: anaplastic lymphoma kinase; dermatan sulphate; ligand; phosphorylation; receptor tyrosine kinase.

Abbreviations: ALK, anaplastic lymphoma kinase; CS, chondroitin sulphate; DS,dermatan sulphate; FCS, foetal calf serum; GAG, glycosaminoglycan; GlcA, glucuronic acid; HP, heparin; HRP, horseradish peroxidase; HS, heparan sulphate; IdoA, iduronic acid; KS, keratan sulphate; NPM, nucleophosmin; NTR, N-terminal region; PTPRσ, phosphatase receptor-type protein-tyrosine phosphatase sigma; RTK, receptor tyrosine kinase; SPR, surface plasmon resonance; TBS, tris-buffered saline.

Anaplastic lymphoma kinase (ALK) is a type I transmembrane protein that belongs to receptor tyrosine kinase (RTK) family, whose intracellular tyrosine kinase activity is enhanced via receptor clustering by specific ligands (*1*). The strong bioactivity of ALK was first discovered when ALK fused to nucleophosmin (NPM-ALK) was produced by t(2; 5)(p23; q35) chromosomal translocation in anaplastic large-cell lymphoma (*2*, *3*). NPM-ALK involves fusion of the C-terminal oligomeric domain of NPM to the kinase domain of ALK, resulting in transphosphorylation and constitutive activation of the tyrosine kinase activity of ALK. Other fusion proteins, such as EML4-ALK and KIF5B-ALK in nonsmall cell lung cancer (*4*, *5*), SQSTM1-ALK in B cell lymphoma (*6*) and STRN-ALK (*7*) in thyroid carcinoma, have also been identified as oncogenic drivers. In addition to these fusion proteins, genomic amplification of ALK or the presence of constitutively active ALK mutants have been found in neuroblastoma (*8–10*) and other tumours. Considering the importance of ALK in malignancy, crizotinib, a first-generation ALK kinase inhibitor, was rapidly developed and approved for clinical application for ALK-driven cancers (*11*, *12*) and received a U.S. Food and Drug Administration Breakthrough Therapy Designation.

ALK is expressed in subsets of developing neurons during the embryonic and postnatal periods of both vertebrates and invertebrates (*13*, *14*). In *Drosophila*, ALK is expressed in the axons of neurons, and regulates axonal projections using its specific ligand Jelly Belly (Jeb) (*15*, *16*). ALK also reportedly regulates presynaptic differentiation in *Caenorhabditis elegans* (*17*). ALK is expressed in the olfactory bulb, thalamus and hippocampus in mice, where it regulates the proliferation of neural progenitor cells and neurotransmitter biosynthesis (*18*). In mice, a recent work reported that ALK was expressed in paraventricular nuclei in the hypothalamus and regulated thinness (*19*); however, ALK knock-out mice were born normally, appeared fertile and showed only slight behavioural deficits (*18*). This variety of results illustrates the difficulties of elucidating the physiological significance of ALK, which has arisen from the absence of established ALK ligands. Although several studies

have reported that the heparin-binding growth factors midkine and pleiotrophin (*20*) are functional ALK ligands (*21*, *22*), subsequent reports failed to reproduce these results (*23*, *24*).

In 2015, Murray *et al*. reported that heparan sulphate (HS) and its over-sulphated moiety heparin (HP) are novel activating ligands for ALK (*25*). HS is a linear sugar chain lacking branches that is classified as a glycosaminoglycan (GAG), along with chondroitin sulphate (CS), dermatan sulphate (DS) and keratan sulphate (KS) (*26*, *27*). GAGs are composed of repeating disaccharide units with heterogeneous sulphation, which appears important for controlling clustering of receptors like RTK. In addition, Murray *et al*., clearly demonstrated a length-dependent regulation of ALK by HS, with an octasaccharide form of HS sufficient for interaction with the basic amino acid cluster found in the N-terminal region (NTR) of ALK, and longer HS forms inducing the clustering and activation of ALK (*25*).

In our previous work, we focussed on the GAG receptor protein-tyrosine phosphatase receptor-type sigma (PTPRσ) that regulated axonal regeneration and its inhibition (*28*). PTPRσ has two GAG ligands, HS and CS (*29*, *30*), and has different biological outputs depending on the bound ligands (*31*). We demonstrated that CS is a signalling ligand that activates PTPRσ and causes the eventual dephosphorylation of cortactin and disruption of autophagy flux (*28*, *32*). Previous findings that HS is an ALK ligand prompted us to investigate whether other GAG moieties are also ligands for ALK.

Materials and Methods

Cell culture

An NB-1 human neuroblastoma cell line with an amplified ALK gene was obtained from the ATCC and maintained in minimum essential medium supplemented with 10% foetal calf serum (FCS) (Biosera) and penicillin–streptomycin (GIBCO). HEK293T cells were purchased from the ATCC and cultured with Dulbecco's modified Eagle medium containing 10% FCS and penicillin–streptomycin. Cell lines were checked for mycoplasma contamination before experiments performed. Plasmid DNA transfections were carried out using Fugene6 (Promega) following the manufacturer's protocol.

Reagents

DS (from pig skin), chondroitin (desulphated product of CS-C) and CS-A (from whale cartilage), -C (from shark cartilage), -D (from shark cartilage) and -E (from squid cartilage) were purchased from the Seikagaku Corporation. DS oligosaccharides of defined length of were purchased from Iduron. These DS oligosaccharides have unsaturated uronic acid (UA) at the nonreducing end because of their production by chondroitinase ABC lyase. Biotinylated DS was obtained from PG research.

Plasmids

The cDNA sequences for human full-length ALK and truncated ALK lacking the NTR were amplified by polymerase chain reaction and cloned into pLVSIN-CMV plasmids (TAKARA).

Surface plasmon resonance (SPR)

SPR analysis was carried out using a BIACORE X-100 (GE Healthcare). The NTR of ALK (Proteintech, Ag21493, 41–366 amino acids encoded by NM_004304) was immobilized onto a CM5 sensor chip (GE Healthcare) by amine-coupling. HBS-EP+ was used as a running buffer. All CS versions were injected at 20 μg/ml for 2 min each. For kinetics analysis, a serial dilution of analytes (0, 1.6, 8, 40, 200 and 1,000 nM) was injected with 5 min final dissociation time at a flowrate of 30 μl/min without regeneration. The sensor chips were regenerated with 2 M NaCl after final analyte injection. Kinetic parameters were calculated based on a 1:1 binding fitting model by BIAevalution software (Cytiva). The molecular weights for all GAGs used in the study were estimated at 10,000.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis and Immunoblotting

Cells were briefly rinsed with tris-buffered saline (TBS) and lysed with TBS containing 1% Triton-X, the Complete protease inhibitor cocktail (Roche) and the Phostop protein phosphatase inhibitor cocktail (Roche). After brief centrifugation, the supernatant was mixed with 4× Laemmli sample buffer (277.8 mM, Tris–HCl, pH 6.8, 44.4% glycerol, 4.4% lithium dodecyl sulphate, 0.02% Bromophenol blue) and heated at 95°C for 3 min. Samples were run on a Supersep 5–20% gradient gel (WAKO) and proteins transferred to nitrocellulose membranes. Membranes were then blocked with 5% nonfat dry milk or Phospho-Blocker (CellBiolab) dissolved in TBS containing 0.1% Tween-20 (TBS-T). The following antibodies were used as the primary antibodies: anti-phospho-ALK (pY1604) (CST, 3341), anti-ALK (CST, 3333) and anti-β-actin (Sigma–Aldrich, clone AC-130). All secondary antibodies were conjugated to horseradish peroxidase (HRP) and purchased from Jackson Immunoresearch. Chemiluminescent signals were produced by reactions with Immobilon Forte Western HRP substrate (Millipore) and images acquired using an AI 680 image analyzer (Amersham). The densitometric analysis was carried out on the band of p220-ALK using ImageJ software.

Immunocytochemistry

NB-1 cells were serum-starved for 18 h then treated with DS at 2.5 μM. After 15 min, cells were briefly washed with phosphate-buffered saline and fixed with 4% paraformaldehyde for 15 min. After fixation, cell membranes were permeabilized with 0.2% Triton-X in PBS for 10 min. Anti-phospho ALK (CST, 3341) was used at 1:200 dilution. The primary antibody allowed to react with the cells overnight. Cells were then incubated with Alexa 594-conjugated anti-rabbit IgG (Invitrogen, A32754) at 1:200 dilution. The secondary antibody allowed to react with the cells for 1 h. Nuclei were then stained with DAPI (DOJINDO). The images were acquired on a BZ-9000 microscope (KEYENCE).

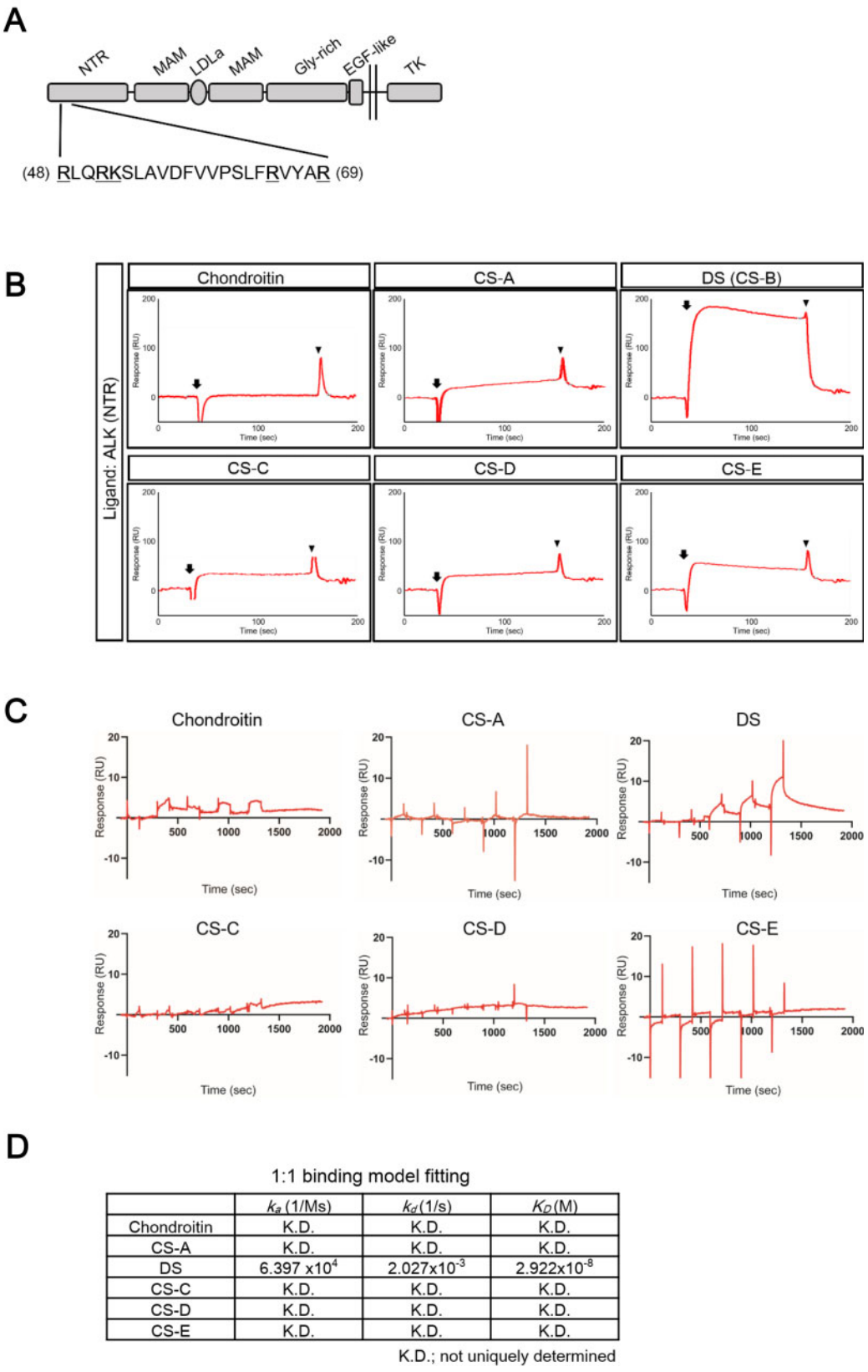
Pull-down of ALK by DS

Full-length ALK and truncated ALK lacking the NTR (ALKΔNTR) were overexpressed in HEK293T cells for 2 days. Cells were lysed and the lysates clarified by centrifugation. Supernatants were incubated with 20 μg of biotinylated DS (PG research) for 1 h at 4°C then incubated with streptavidin-agarose beads for 1 h at 4°C. Beads were next washed three times with TBS containing 1% Triton-X and the proteins were eluted using 2× Laemmli sample buffer.

Results and Discussion

The basic amino acid cluster on the NTR of ALK has been reported to interact with HS via an ionic bond (Fig. 1A). We hypothesized that this region might be important for interactions with other sulphated glycans containing negative charges, especially CS. To address this possibility, SPR analysis was carried out. The NTR of ALK was immobilized on a carboxymethyl sensor chip and solutions of CS and DS with different sulphation patterns (20 μg/ml) were injected as analytes. As shown in Fig. 1B, DS (also known as CS-B) showed significant interaction with the NTR of ALK while CS forms containing other sulphation patterns did not bind to ALK. The single cycle kinetics for the interaction of these glycans and ALK again showed significant interaction between DS and ALK at 2.922×10^{-8} M of dissociate constant (K_D) (Fig. 1C and D).

To address whether or not DS activates ALK in living cells, the human neuroblastoma cell line NB-1 was used due to the high expression of wild-type ALK



	k_a (1/Ms)	k_d (1/s)	K_D (M)
Chondroitin	K.D.	K.D.	K.D.
CS-A	K.D.	K.D.	K.D.
DS	6.397×10^{4}	2.027×10^{-3}	2.922×10^{-8}
CS-C	K.D.	K.D.	K.D.
CS-D	K.D.	K.D.	K.D.
CS-E	K.D.	K.D.	K.D.

K.D.; not uniquely determined

Fig. 1. DS binds to ALK. (**A**) The structure of ALK, with the basic amino acid cluster (amino acids 48–69) of the NTR highlighted. MAM: meprin, A-5 protein, and receptor protein-tyrosine phosphatase mu domain. LDL-a: low-density lipoprotein receptor class A domain. Gly-rich: glycine rich domain. EGF-like: epidermal growth factor-like domain. TK: tyrosine kinase domain. (**B**) SPR analysis. The indicated analyte (20 μg/ml) was injected onto a sensor chip containing immobilized ALK (NTR) sensor chip at the arrow to the arrowhead. Flow; 20 μl/min. (**C**) Kinetics analysis for the interaction between ALK (NTR) and CS or DS. The indicated analyte (0, 1.6, 8, 40, 200 and 1,000 nM) was continuously injected without regeneration at 30 μl/min. (**D**) The binding parameters were calculated at 1:1 binding fitting model. The molecular weights of all GAGs were estimated as 10,000.

protein via genomic amplification in NB-1 cells. Cells were serum-starved and treated with DS of a particular length for 15 min. As shown in Fig. 2A–D, DS oligosaccharides longer than dp4 induced autophosphorylation of ALK with 220 kDa of molecular weight (p220 ALK) at Y1604 in a dose-dependent manner (Fig. 2F), which produces activate ALK. Similar results were obtained using DS polysaccharide from pig skin, although it did not activate ALK at 5 μM (Fig. 2E). Importantly, longer oligosaccharides induced stronger autophosphorylation of ALK, suggesting that the longer DS oligosaccharides tend to enhance clustering of ALK, as is the case for heparin oligosaccharide (Fig. 2H). Of note, identical concentrations of DS dp16 and HP dp 20 induced activation of ALK at similar levels (Fig. 2I). The 140 kDa form of ALK (p140-ALK) is the truncated ALK which is produced by an alternative transcription initiation, extracellular cleavage or results of alternative splicing. Some of these isoforms were reported to lack the NTR and some are still yet uncharacterized (*9*, *33*, *34*). In our experiments, the isoform also seemed to be phosphorylated with some DS especially at high concentration (Fig. 2B–D). Even though further study is still needed, we concluded that the NTR was indeed the major specific binding site for DS. Immunocytochemical analysis on NB-1 cells also supported that DS was ligands for ALK (Fig. 3).

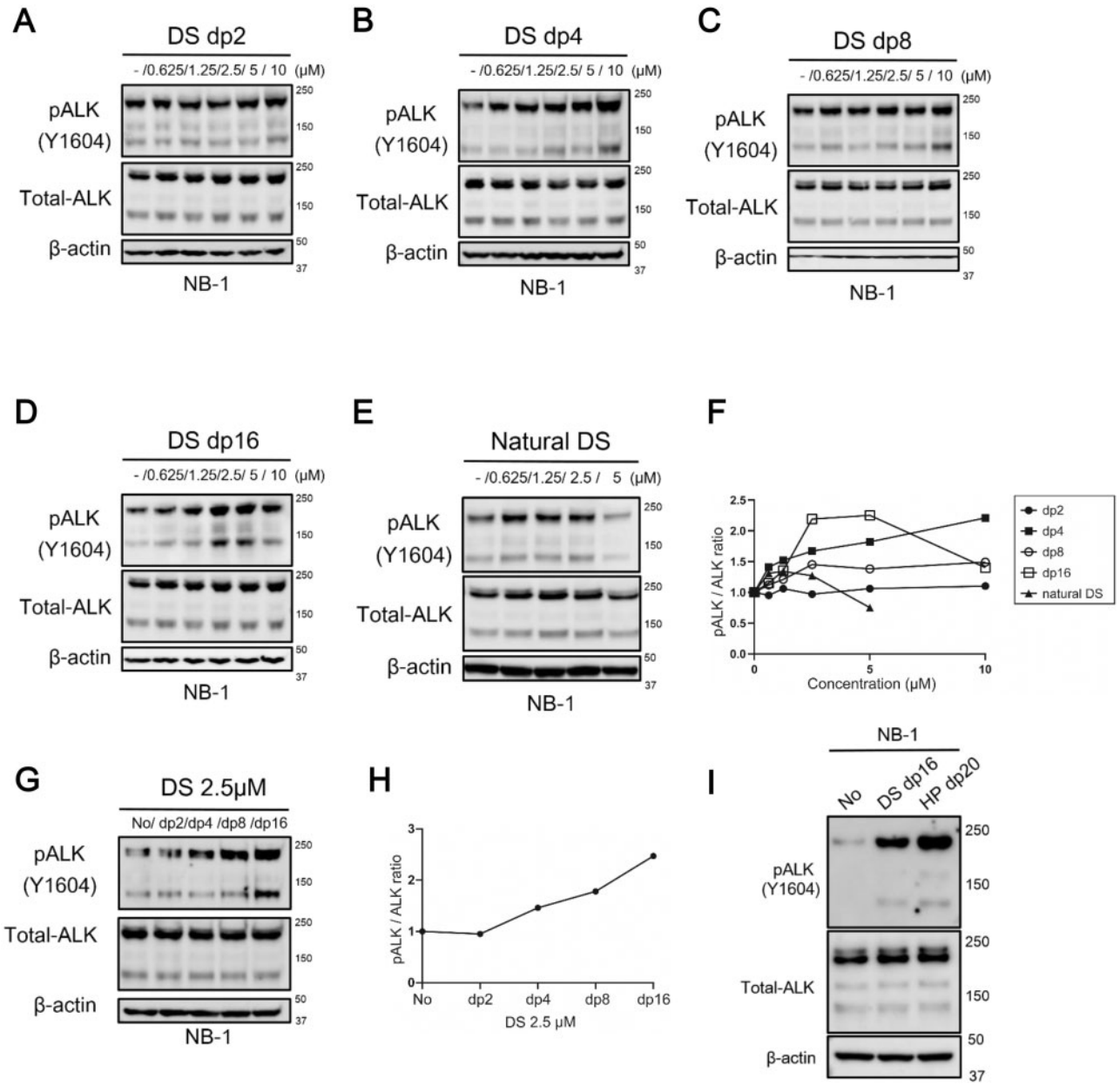


Fig. 2. Activation of cellular ALK by DS. (**A**–**E**) NB-1 cells were serum-starved for 18 h and stimulated with the increasing concentration of the specified of DS oligosaccharide (A to D) or DS polysaccharides from natural sources (E) for 15 min. Except for DS dp2, DS oligosaccharides induced autophosphorylation of ALK. (**F**) The densitometric analysis of A to E. Total and phosphorylated p220-ALK were analysed. (**G**) NB-1 cells were stimulated with 2.5 μM of DS oligosaccharides for 15 min, with longer oligosaccharides inducing stronger activation of ALK. (**H**) The densitometric analysis of G. Total and phosphorylated p220-ALK were analysed. (**I**) NB-1 cells stimulated with the same concentration of either DS dp16 or HP (heparin) dp20. dp; degree of polymerization.

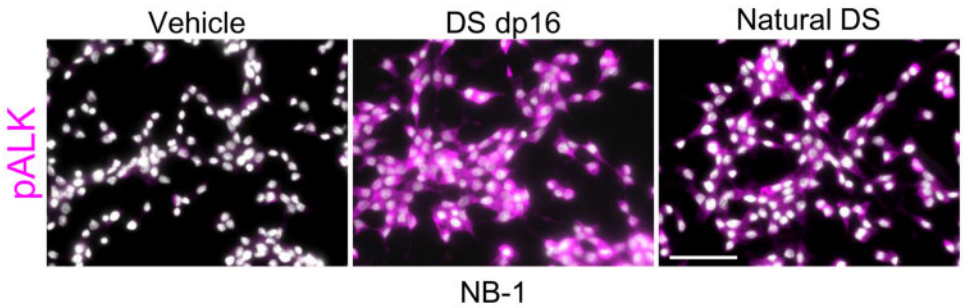


Fig. 3. Immunocytochemical analysis of pALK in NB-1. NB-1 cells were stimulated with the same concentration (2.5 μM) of either DS oligosaccharides or natural DS for 15 min. Bar: 20 μm.

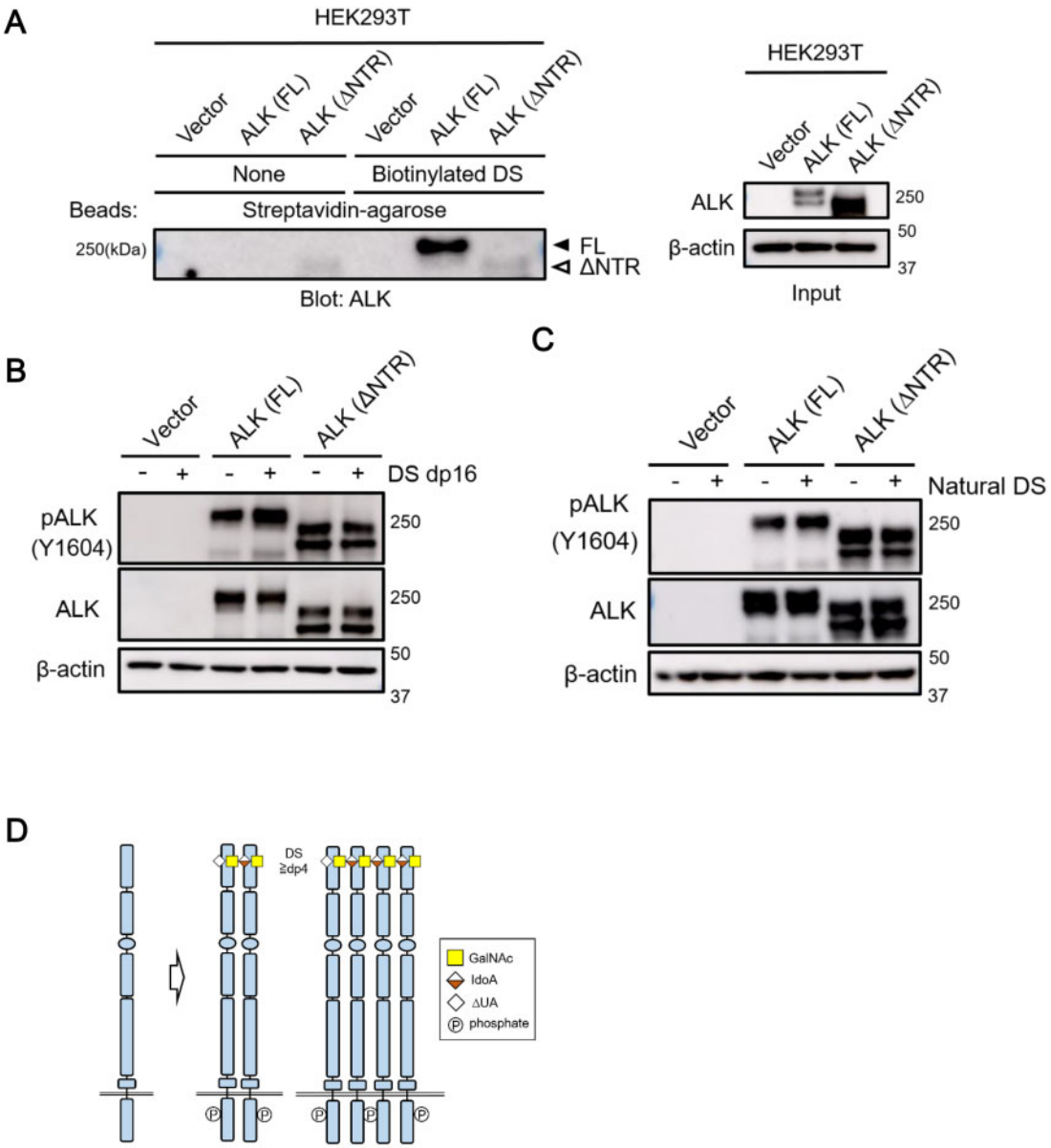


Fig. 4. The NTR is crucial for binding of DS and DS-mediated activation of ALK. (**A**) Full-length ALK (FL) but not ALK lacking the N-terminal domain (ΔNTR) interacted with DS. Both ALK (FL) and ALK (ΔNTR) were expressed in HEK293T cells. Cell lysates were added to affinity pull-down columns containing biotinylated DS and streptavidin-agarose. Black arrowheads: the location of ALK (FL), white arrowheads: the location of ΔNTR. (**B**) HEK293T cells overexpressing ALK (FL) and ΔNTR were treated with 2.5 μM DS dp16 for 15 min. (**C**) HEK293T cells overexpressing ALK (FL) or ΔNTR and treated with 2.5 μM natural DS at for 15 min. (**D**) A schematic summary of the results, in which DS molecules larger than DS tetrasaccharides (dp4) appear to induce ALK clustering and activation. The NTR of ALK is a potent binding site for DS. GalNAc: *N*-acetylgalactosamine. IdoA: Iduronic acid. ΔUA: unsaturated uronic acid with C4–C5 double bond.

To further address the significance of the NTR of ALK, we expressed full-length ALK and truncated ALK lacking the NTR (ALKΔNTR). Afterward, cellular lysates were mixed with biotin-conjugated DS and pulled down with streptavidin. As shown in Fig. 4A, full-length ALK was successfully pulled down by DS, confirming that DS is a specific ligand for ALK. In contrast, the truncated ALKΔNTR failed to be affinity-precipitated by DS, supporting the idea that the NTR on ALK was a binding site for ALK. In addition, DS dp16 and DS from pig skin consistently could not induce activation of ALKΔNTR, while they activated the full-length ALK (Fig. 4B and C).

As described above, we showed that DS is a specific ligand for ALK. DS shares an ALK binding site with HS, and the longer DS oligosaccharides displayed higher activity than HS (*25*). As discussed above, while HS octasaccharides were sufficient for ALK activation, DS tetrasaccharides with unsaturated UA at the nonreducing end were sufficient for ALK activation (Fig. 4D). Interestingly, CS did not show any affinity for ALK. The difference between DS and CS is the form of UA present, with DS containing iduronic acid (IdoA) and CS containing glucuronic acid (GlcA). Remarkably, some portions of HS contain IdoA as their UA component. Our results therefore suggest that IdoA may be a key component for recognition by ALK.

DS is displayed on core protein and expressed as a proteoglycan such as decorin and biglycan. They are enriched in the skin, blood vessels, bone and nervous systems (*35*). For example, in the skin, DS regulates collagen metabolism and turnover and dermatan sulphate 4 sulfotransferase-1, a DS-modifying enzyme, is reportedly involved in Ehlers–Danlos syndrome, a hereditary connective tissue disorder characterized by hyperextensible skin (*36*). In addition to these matrix remodelling functions, our current results provide insights into the role of DS as a signalling molecule activating ALK.

Conclusion

We have reported that DS is a new ligand of ALK. DS directly interacted with the NTR of ALK and the DS tetrasaccharide was sufficient to induce ALK activation.

Author Contribution

M.M., Y.G., T.O., Y.S., E.W., S.I. and K.S. carried out the experiments. K.K and K.S. wrote the manuscript.

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Conflict of Interest

None declared.

References

1. Hallberg, B. and Palmer, R.H. (2013) Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. *Nat. Rev. Cancer* **13**, 685–700
2. Morris, S.W., Kirstein, M.N., Valentine, M.B., Dittmer, K.G., Shapiro, D.N., Saltman, D.L., and Look, A.T. (1994) Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* **263**, 1281–1284
3. Shiota, M., Fujimoto, J., Semba, T., Satoh, H., Yamamoto, T., and Mori, S. (1994) Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. *Oncogene* **9**, 1567–1574
4. Rikova, K., Guo, A., Zeng, Q., Possemato, A., Yu, J., Haack, H., Nardone, J., Lee, K., Reeves, C., Li, Y., Hu, Y., Tan, Z., Stokes, M., Sullivan, L., Mitchell, J., Wetzel, R., Macneill, J., Ren, J.M., Yuan, J., Bakalarski, C.E., Villen, J., Kornhauser, J.M., Smith, B., Li, D., Zhou, X., Gygi, S.P., Gu, T.L., Polakiewicz, R.D., Rush, J., and Comb, M.J. (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* **131**, 1190–1203
5. Soda, M., Choi, Y.L., Enomoto, M., Takada, S., Yamashita, Y., Ishikawa, S., Fujiwara, S., Watanabe, H., Kurashina, K., Hatanaka, H., Bando, M., Ohno, S., Ishikawa, Y., Aburatani, H., Niki, T., Sohara, Y., Sugiyama, Y., and Mano, H. (2007) Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* **448**, 561–566
6. Takeuchi, K., Soda, M., Togashi, Y., Ota, Y., Sekiguchi, Y., Hatano, S., Asaka, R., Noguchi, M., and Mano, H. (2011) Identification of a novel fusion, SQSTM1-ALK, in ALK-positive large B-cell lymphoma. *Haematologica* **96**, 464–467
7. Kelly, L.M., Barila, G., Liu, P., Evdokimova, V.N., Trivedi, S., Panebianco, F., Gandhi, M., Carty, S.E., Hodak, S.P., Luo, J., Dacic, S., Yu, Y.P., Nikiforova, M.N., Ferris, R.L., Altschuler, D.L., and Nikiforov, Y.E. (2014) Identification of the transforming STRN-ALK fusion as a potential therapeutic target in the aggressive forms of thyroid cancer. *Proc. Natl. Acad. Sci. USA* **111**, 4233–4238
8. Chen, Y., Takita, J., Choi, Y.L., Kato, M., Ohira, M., Sanada, M., Wang, L., Soda, M., Kikuchi, A., Igarashi, T., Nakagawara, A., Hayashi, Y., Mano, H., and Ogawa, S. (2008) Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* **455**, 971–974
9. George, R.E., Sanda, T., Hanna, M., Fröhling, S., Luther, W., Zhang, J., Ahn, Y., Zhou, W., London, W.B., McGrady, P., Xue, L., Zozulya, S., Gregor, V.E., Webb, T.R., Gray, N.S., Gilliland, D.G., Diller, L., Greulich, H., Morris, S.W., Meyerson, M., and Look, A.T. (2008) Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* **455**, 975–978
10. Mossé, Y.P., Laudenslager, M., Longo, L., Cole, K.A., Wood, A., Attiyeh, E.F., Laquaglia, M.J., Sennett, R., Lynch, J.E., Perri, P., Laureys, G., Speleman, F., Kim, C., Hou, C., Hakonarson, H., Torkamani, A., Schork, N.J., Brodeur, G.M., Tonini, G.P., Rappaport, E., Devoto, M., and Maris, J.M. (2008) Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* **455**, 930–935
11. Christensen, J.G., Zou, H.Y., Arango, M.E., Li, Q., Lee, J.H., McDonnell, S.R., Yamazaki, S., Alton, G.R., Mroczkowski, B., and Los, G. (2007) Cytoreductive antitumor activity of PF-2341066, a

novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. *Mol. Cancer Ther.* **6**, 3314–3322
12. Kwak, E.L., Bang, Y.J., Camidge, D.R., Shaw, A.T., Solomon, B., Maki, R.G., Ou, S.H., Dezube, B.J., Jänne, P.A., Costa, D.B., Varella-Garcia, M., Kim, W.H., Lynch, T.J., Fidias, P., Stubbs, H., Engelman, J.A., Sequist, L.V., Tan, W., Gandhi, L., Mino-Kenudson, M., Wei, G.C., Shreeve, S.M., Ratain, M.J., Settleman, J., Christensen, J.G., Haber, D.A., Wilner, K., Salgia, R., Shapiro, G.I., Clark, J.W., and Iafrate, A.J. (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N. Engl. J. Med.* **363**, 1693–1703
13. Iwahara, T., Fujimoto, J., Wen, D., Cupples, R., Bucay, N., Arakawa, T., Mori, S., Ratzkin, B., and Yamamoto, T. (1997) Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene* **14**, 439–449
14. Vernersson, E., Khoo, N.K., Henriksson, M.L., Roos, G., Palmer, R.H., and Hallberg, B. (2006) Characterization of the expression of the ALK receptor tyrosine kinase in mice. *Gene Expr. Patterns* **6**, 448–461
15. Bazigou, E., Apitz, H., Johansson, J., Lorén, C.E., Hirst, E.M., Chen, P.L., Palmer, R.H., and Salecker, I. (2007) Anterograde Jelly belly and Alk receptor tyrosine kinase signaling mediates retinal axon targeting in *Drosophila*. *Cell* **128**, 961–975
16. Lee, H.H., Norris, A., Weiss, J.B., and Frasch, M. (2003) Jelly belly protein activates the receptor tyrosine kinase Alk to specify visceral muscle pioneers. *Nature* **425**, 507–512
17. Liao, E.H., Hung, W., Abrams, B., and Zhen, M. (2004) An SCF-like ubiquitin ligase complex that controls presynaptic differentiation. *Nature* **430**, 345–350
18. Bilsland, J.G., Wheeldon, A., Mead, A., Znamenskiy, P., Almond, S., Waters, K.A., Thakur, M., Beaumont, V., Bonnert, T.P., Heavens, R., Whiting, P., McAllister, G., and Munoz-Sanjuan, I. (2008) Behavioral and neurochemical alterations in mice deficient in anaplastic lymphoma kinase suggest therapeutic potential for psychiatric indications. *Neuropsychopharmacology* **33**, 685–700
19. Orthofer, M., Valsesia, A., Mägi, R., Wang, Q.P., Kaczanowska, J., Kozieradzki, I., Leopoldi, A., Cikes, D., Zopf, L.M., Tretiakov, E.O., Demetz, E., Hilbe, R., Boehm, A., Ticevic, M., Nõukas, M., Jais, A., Spirk, K., Clark, T., Amann, S., Lepamets, M., Neumayr, C., Arnold, C., Dou, Z., Kuhn, V., Novatchkova, M., Cronin, S.J.F., Tietge, U.J.F., Müller, S., Pospisilik, J.A., Nagy, V., Hui, C.C., Lazovic, J., Esterbauer, H., Hagelkruys, A., Tancevski, I., Kiefer, F.W., Harkany, T., Haubensak, W., Neely, G.G., Metspalu, A., Hager, J., Gheldof, N., and Penninger, J.M. (2020) Identification of ALK in thinness. *Cell* **181**, 1246–1262.e1222
20. Sakamoto, K. and Kadomatsu, K. (2012) Midkine in the pathology of cancer, neural disease, and inflammation. *Pathol. Int.* **62**, 445–455
21. Stoica, G.E., Kuo, A., Powers, C., Bowden, E.T., Sale, E.B., Riegel, A.T., and Wellstein, A. (2002) Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. *J. Biol. Chem.* **277**, 35990–35998
22. Stoica, G.E., Kuo, A., Aigner, A., Sunitha, I., Souttou, B., Malerczyk, C., Caughey, D.J., Wen, D., Karavanov, A., Riegel, A.T., and Wellstein, A. (2001) Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. *J. Biol. Chem.* **276**, 16772–16779
23. Mathivet, T., Mazot, P., and Vigny, M. (2007) In contrast to agonist monoclonal antibodies, both C-terminal truncated form and full length form of Pleiotrophin failed to activate vertebrate ALK (anaplastic lymphoma kinase)? *Cell Signal.* **19**, 2434–2443
24. Moog-Lutz, C., Degoutin, J., Gouzi, J.Y., Frobert, Y., Brunet-de Carvalho, N., Bureau, J., Créminon, C., and Vigny, M. (2005) Activation and inhibition of anaplastic lymphoma kinase receptor tyrosine kinase by monoclonal antibodies and absence of agonist activity of pleiotrophin. *J. Biol. Chem.* **280**, 26039–26048
25. Murray, P.B., Lax, I., Reshetnyak, A., Ligon, G.F., Lillquist, J.S., Natoli, E.J., Shi, X., Folta-Stogniew, E., Gunel, M., Alvarado, D., and Schlessinger, J. (2015) Heparin is an activating ligand of the orphan receptor tyrosine kinase ALK. *Sci. Signal.* **8**, ra6
26. Kadomatsu, K. and Sakamoto, K. (2014) Mechanisms of axon regeneration and its inhibition: roles of sulfated glycans. *Arch. Biochem. Biophys.* **558**, 36–41
27. Kadomatsu, K. and Sakamoto, K. (2014) Sulfated glycans in network rewiring and plasticity after neuronal injuries. *Neurosci. Res.* **78**, 50–54
28. Sakamoto, K., Ozaki, T., Ko, Y.C., Tsai, C.F., Gong, Y., Morozumi, M., Ishikawa, Y., Uchimura, K., Nadanaka, S., Kitagawa, H., Zulueta, M.M.L., Bandaru, A., Tamura, J.I., Hung, S.C., and Kadomatsu, K. (2019) Glycan sulfation patterns define autophagy flux at axon tip via PTPRsigma-cortactin axis. *Nat. Chem. Biol.* **15**, 699–709
29. Aricescu, A.R., McKinnell, I.W., Halfter, W., and Stoker, A.W. (2002) Heparan sulfate proteoglycans are ligands for receptor protein tyrosine phosphatase sigma. *Mol. Cell. Biol.* **22**, 1881–1892
30. Shen, Y., Tenney, A.P., Busch, S.A., Horn, K.P., Cuascut, F.X., Liu, K., He, Z., Silver, J., and Flanagan, J.G. (2009) PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. *Science* **326**, 592–596
31. Coles, C.H., Shen, Y., Tenney, A.P., Siebold, C., Sutton, G.C., Lu, W., Gallagher, J.T., Jones, E.Y., Flanagan, J.G., and Aricescu, A.R. (2011) Proteoglycan-specific molecular switch for RPTPσ clustering and neuronal extension. *Science* **332**, 484–488
32. Tran, A.P., Warren, P.M., and Silver, J. (2020) Regulation of autophagy by inhibitory CSPG interactions with receptor PTPσ and its impact on plasticity and regeneration after spinal cord injury. *Exp. Neurol.* **328**, 113276
33. Huang, H. (2018) Anaplastic Lymphoma Kinase (ALK) receptor tyrosine kinase: a catalytic receptor with many faces. *Int. J. Mol. Sci.* **19**, 3448.
34. Souttou, B., Carvalho, N.B., Raulais, D., and Vigny, M. (2001) Activation of anaplastic lymphoma kinase receptor tyrosine kinase induces neuronal differentiation through the mitogen-activated protein kinase pathway. *J. Biol. Chem.* **276**, 9526–9531
35. Trowbridge, J.M. and Gallo, R.L. (2002) Dermatan sulfate: new functions from an old glycosaminoglycan. *Glycobiology* **12**, 117R–125R
36. Dündar, M., Müller, T., Zhang, Q., Pan, J., Steinmann, B., Vodopiutz, J., Gruber, R., Sonoda, T., Krabichler, B., Utermann, G., Baenziger, J.U., Zhang, L., and Janecke, A.R. (2009) Loss of dermatan-4-sulfotransferase 1 function results in adducted thumb-clubfoot syndrome. *Am. J. Hum. Genet.* **85**, 873–882