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Exploiting the features of short peptides to recognize specific cell surface markers

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Introduction

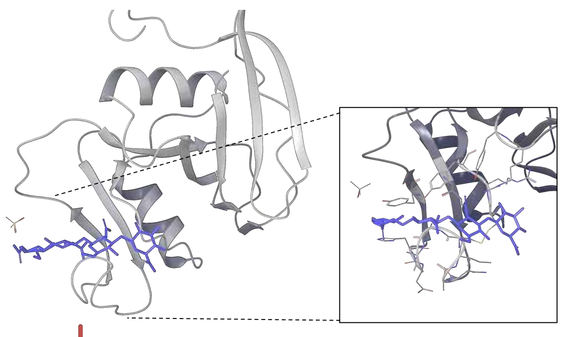
Stem cells have an unlimited potential for cell division and the ability to transdifferentiate into other cell types. In recent years they grew to be a first-line weapon of regenerative medicine for repairing tissue and organic abnormalities caused by diseases, congenital deficiencies, and age-related effects[1]. Bone marrow-derived mesenchymal stem cells (MSCs) mainly develop in adipocytes, chondroblasts, and osteoblasts but also show the property to successfully transdifferentiate in neural, myocyte, and epidermal cells when engrafted in endogenous tissues under specific conditions[2]. Efficient isolation of MSCs from other cell components in the bone marrow is fundamental to obtaining valuable MSC clinical applications. Currently, the most widespread purification methods include the isolation of MSCs based on the expression of precise surface markers, thus involving specific antibodies[3]. In this work, we aimed to develop a fast and affordable method for the phenotypic characterization and isolation of MSCs from bone marrow without using immunoglobulins. To this purpose, we designed short peptides based on the deposited 3D structures of two specific MSCs surface markers, CD44 and CD271, in complex with their natural ligands. The peptides were selected by computational modeling, synthesized by solid-phase peptide synthesis, and tested in cell assays. Interestingly the data show that starting from a small set of peptides, using a protocol based on molecular simulations, it is possible to obtain small ligands endowed with a significant specificity in targeting CD44 and CD271 in place of their antibody, bypassing the common challenges and issues due to the use of immunoglobulins.

Results

Computational modelling

CD44

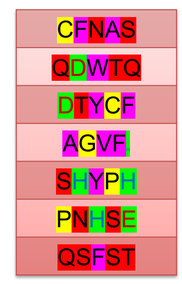
PDB ID: 4MRD
Residues: 23-173
Organism: Mus musculus
Ligand: Hyaluronic acid (HA) (in blue)



Residues involved in binding with HA:
R45 Y46 C81 R82 Y83 I92 N98 A99 I100 C101 A102 A103 N104 H105 Y109

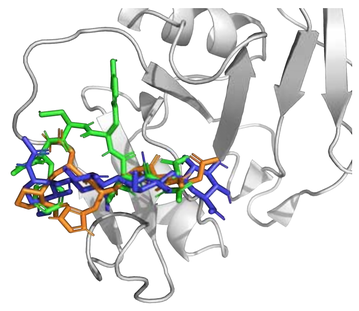
Grid box built on these residues

Screening of 250 random peptide sequences



- Polar uncharged amino acids
- Hydrophobic amino acids
- Positively charged amino acids
- Negatively charged amino acids
- Special amino acids

Redocking →

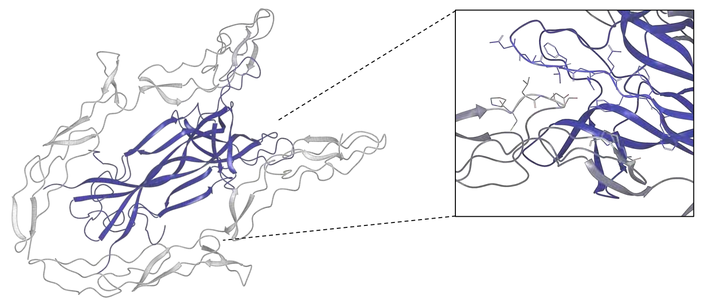


- HA
- DTYCF
- PNHSE

Ligand	HA	DTYCF	PNHSE
Docking score (kJ/mol)	-10.3	-9.6	-8.6

CD271

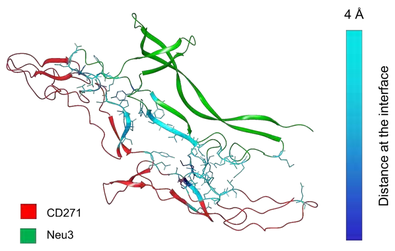
PDB ID: 3IJ2
Residues: 30-190
Organism: Rattus norvegicus
Ligand: Neurotrophin 3 (Neu3) (in blue)



Residues involved in binding with Neu3:
P67 C68 L69 D70 N71 M96 P99

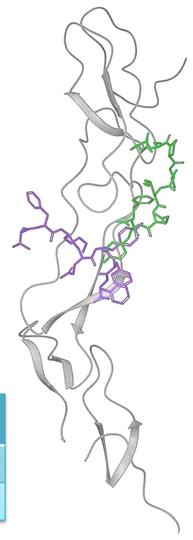
Grid box built on these residues

Peptide sequences selected from Neu3 residues interacting with CD271.



VFQYFF
RGEFSVCD
DGKQAAWRF
NSPVKQYF
KSHRGEY
KEARPVK
HMGEF
DSVSVW
SVFRQYFF
RGIDSKHW
QAAWRF

- NSPVKQYF
- VFQYFF



Ligand	Score (kJ/mol)	Poses with RMSD < 5 (out of 100)
VFQYFF	-7.3	18
NSPVKQYF	-8.1	6

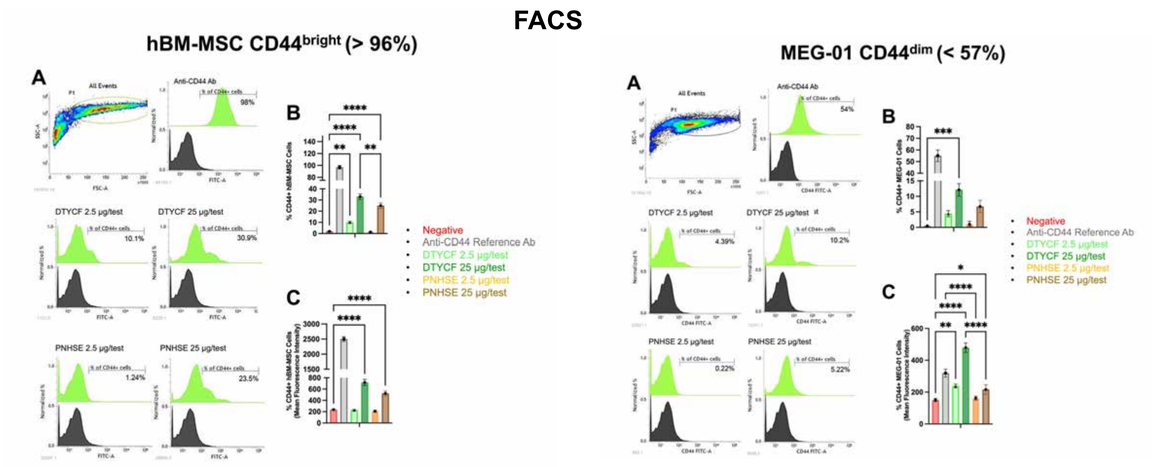
Solid Phase Peptide Synthesis

DTYCF, PNHSE, NSPVKQYF and VFQYFF were synthesized by classical Merrifield SPPS[4] using the N-α-fluorenylmethoxycarbonyl-protected amino acids and a Rink-amide resin as a solid support.
In order to detect these peptides for FACS assays, the same sequences were synthesized, attaching as the last residue in the C->N peptide synthesis a fluorophore tag; we used the 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) conjugated to the 2,3-Diaminopropionic acid (Dap). NBD excitation is at 480 nm and the emission is at 540 nm.

Cell assays

CD44

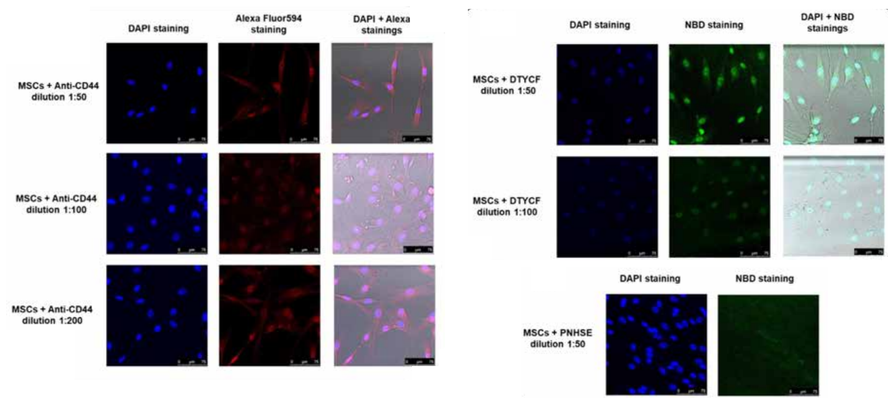
FACS



Cytofluorometric analysis of h-BM-MSC CD44^{bright} and MEG-01 CD44^{dim} cells stained with NBD-DTYCF, NBD-PNHSE or FITC-Anti-CD44 Ab as control. Panel (**A**) reports a representative FACS histogram profile of surface staining with reference Ab (upper right) and the two peptides (middle and bottom) at different concentrations. Black-filled curves referred to the negative control cells. Bars graph in panels (**B**,**C**) report, respectively, the % ± SD of fluorescent positive cells and mean fluorescence intensity (MFI) values ± SD on viable gated cells. Pairwise comparisons statistically significant are indicated (ANOVA; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$).

The dose-response increase in fluorescence proportional to the expression of the CD44 marker might suggest that both DTYCF and PNHSE peptides can bind the surface receptor CD44.

Immunostaining



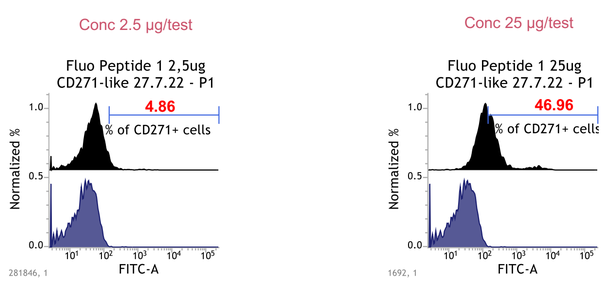
Immunostaining results: On the left, DAPI-stained human bone marrow mesenchymal stem cells (MSCs) with Alexa Fluor594 stained anti-CD44 in three different dilution ratios (1:50, 1:100, 1:200); on the top right, MSCs with NBD conjugated DTYCF in two different dilution ratios (1:50, 1:100); on the bottom right, MSCs with NBD conjugated PNHSE in 1:50 dilution ratio.

These data support a more significant interaction of DTYCF with MSCs compared to PNHSE, confirming the results we previously collected in the FACS experiments.

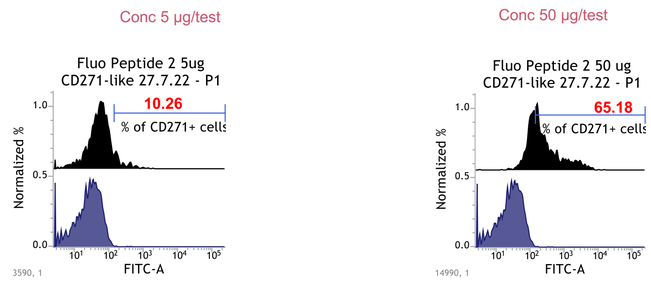
CD271

FACS – preliminary results

NSPVKQYF



VFQYFF



→ ***Work in progress***

Conclusions. In this poster, we report a methodology for the use of short peptide sequences as a tool for phenotypic characterization and the isolation of cell bodies, exploiting their binding of surface cell markers. Taken together, the data show that using an ad hoc designed computational protocol, it is possible to obtain short peptide sequences with adequate specificity to study the expression of the CD44 and CD271 receptors by an antibody-free assay. Replacing antibodies with short peptides can be a viable strategy in all procedures that require the use of antibodies to preserve sensitivity and specificity while avoiding high costs and complex laboratory protocols. This work paves the way towards the identification of a range of peptide aptamers targeting biomarkers relevant to existing or emerging biomedical applications such as regenerative medicine. In the latter case, the availability of these aptamers will enable the selection of specific cell populations suitable for tissue regeneration as such or in combination with nanoparticles.

References: [1]Mason, C.; Dunnill, P. A brief definition of regenerative medicine. Future Medicine 2008, 3; [2]Samsonraj, R.M.; Raghunath, M.; Nurcombe, V.; Hui, J.H.; van Wijnen, A.J.; Cool, S.M. Concise review: multifaceted characterization of human mesenchymal stem cells for use in regenerative medicine. Stem cells translational medicine 2017, 6, 2173-2185; [3]Pittenger, M.F. Multilineage Potential of Adult Human Mesenchymal Stem Cells. Science 1999, 284, 143-147.[4]Merrifield, Robert B. "Solid phase peptide synthesis. I. The synthesis of a tetrapeptide." Journal of the American Chemical Society 85.14 (1963): 2149-2154.