



Title	Regulation of Nectin-2 by Cadmium Chloride (CdCl₂)
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Structural and biochemical analysis of a novel, broadly specific claudin binder.

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Objective: Recent progress in understanding the biochemical structure of tight junctions has provided us with new insights for claudin (CL)-targeted drug development. CLs are a family of tetra-transmembrane proteins comprising over 24 members. CL binders enhance mucosal absorption of drugs, so CL targeting is a promising strategy for mucosal vaccination and cancer therapy. Previously, by using a library of mutated C-terminal fragments of *Clostridium perfringens* enterotoxin (C-CPE), we screened a CL binder, m19. Here, we performed structural and biochemical analyses of m19 and its mutants.

Methods: X-ray diffraction data on m19 were collected from a single crystal at 2.0 Å by using the BL44XU beamline at SPring-8, and processed with iMOSFLM and SCALA software. The structure of m19 was solved by using the BALBES system. The interactions of m19 and its mutants with CLs were investigated with ELISA and FACS analysis.

Results: C-CPE binds to CLs via interaction between the second extracellular loop domain of the CL and the 30 C-terminal amino acids of C-CPE. The structural backbone of m19 in the C-terminal domain is similar to that of C-CPE. An electrostatic surface map of m19 revealed that its C-terminal domain is more positively charged than that of C-CPE. Kimura et al. proposed an electrostatic interaction model for the binding of CPE and CLs (J. Biol. Chem., 285, 401, 2010) and showed that part of the second loop domain interacts with the CL-binding domain of C-CPE. The isoelectric point (pI) of CL-4 is higher than that of CL-1, -2, and -5, which interact less with CPE. Therefore, we hypothesized that if m19 binds to CL-1 via electric interaction, it would also bind to CL-2 and -5. m19 bound to CL-1, -2, -4, and -5-expressing cells; therefore, m19 is a broadly specific CL binder. Site-directed analyses showed that substitution of Ser at position 307 and Ser at position 313 with Arg and His, respectively, may be essential for the interaction with CL-1. We prepared double-substituted C-CPE mutants by changing the Ser (pI, 5.68) residue to His (pI, 7.59), Lys (pI, 9.8), or Arg (pI, 10.76). Increase in positive charge at position 307 and in negative charge at position 313, respectively, strengthened the interaction with CL-1. We also prepared double Ala (pI, 6.0)- or Asp (pI, 2.77)-substituted C-CPE mutants at positions 307 and 313 (S307A/S313A and S307D/S313D). The decrease in the charge attenuated the binding to CL-1.

Conclusion: Electrostatic interactions may be involved in the binding of m19 to CL-1. Therefore, modulation of the electrostatic surface may be a potent strategy for the development of CL binders.

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Regulation of Nectin-2 by Cadmium Chloride (CdCl₂).

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Nectin-2 is a major component of the adherens junctions (AJs) between Sertoli cells and germ cells in the testis. Recent studies have shown that male knockout mice of nectin-2 are sterile. Cadmium (Cd), an environmental toxicant, is known to be also an endocrine disruptor that affects spermatogenesis. In this study, we investigate whether cadmium chloride (CdCl₂) plays a

role in nectin-2 expression. CdCl₂ negatively regulates mRNA and protein levels of nectin-2 in mouse Sertoli cell line, TM4 cells. Luciferase reporter assays indicated that CdCl₂ reduces nectin-2 promoter activity within the region of nucleotides (nt) -246 and -211 (relative to the translation start site) where putative transcription factors (TFs) binding motifs are identified. However, site-directed mutational studies have shown that no specific motif is found to involve in CdCl₂-mediated nectin-2 gene repression. Hence, six consecutive cis-acting regions (each contains 6 nucleotides) between nt -246 and -211 are mutated respectively to identify the cis-acting region involved in the CdCl₂ effect. Results showed that the second 6-bp region (between nt -240 and -235) is involved in CdCl₂-mediated reduction of nectin-2 promoter activity. In addition, putative TFs binding to this region are identified. By EMSAs, we found that DNA (nt -240 to -235)-protein complexes are formed in a dose-dependent manner and CdCl₂ treatment could diminish the formation of the complexes. Antibody supershift assays have shown that TFs, E2F1, Sp1 and KLF4, are present in the complexes. We also found that CdCl₂ down-regulates the expression level of these TFs including E2F1, Sp1 and KLF4 in the nucleus. Apart from transcriptional regulation, cycloheximide assay indicated that CdCl₂ negatively modulates nectin-2 protein level via post-translational modification and we are now investigating the underlying mechanism. Taken together, CdCl₂-mediated down-regulation of nectin-2 is mediated through transcriptional modification by negatively affecting its basal gene transcription and post-translational modification. [This work was supported by Hong Kong Research Grants Council (HKU772009 and HKU773710) and CRCG Seed Funding for Basic Research.]

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MHC Class II Compartment in Human Autologous Macrophage-Lymphocyte Rosettes.

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The contact area between a T cell and antigen-presenting cell is known as "immunological synapse" (Grakoui et al, 1999) and the multiple interactions that occur leading to a "signal" for T cell activation. Cellular association between human blood monocyte-derived macrophages and lymphocytes T CD4+ from autologous cultures total leukocytes extracted from the blood, which bind selectively forming rosettes with central macrophage and lymphocytes adhered was described as the phenomenon of multiple immunological synapses on macrophage-lymphocyte rosette (MLR) (Cabral and Novak, 1992, 99). The processing and antigen presentation are involved in MLR phenomenon (Cabral and Novak, 1999, Novak and Cabral 2008, 09) and in this phenomenon the autologous antigens of senescent neutrophils are presented by endocytic way like phagocytosis of cells undergoing apoptosis naturally in the body. Along time of culture the cells that interact in the MLR phenomenon may present special features in their areas of cell-cell interaction, and in the surrounding cytoplasm such as mitochondrial translocation (Novak y Orquera, 2011). The spatial organization of MHC class II compartment (MIIC) changes in dendritic cells maturation with morphological characteristic such as multivesicular, multilaminar and tubular structures (Van Nispen tot Pannerden et al, 2010). Objectives: ultrastructural study to observe the organization of MIIC in the cells of the MLRs along culture time. Materials and methods: Healthy human blood samples, anticoagulated with heparin (n = 10) (donated by the Blood Bank, UNC, anonymity, data serology). Autologous cultures in TC199 medium (SIGMA, St. Louis, MO). Samples: 1, 2, 3, 20, 48, 96 and 144 h. MLR technique (Cabral y Novak, 1992, 99). Samples of MLRs underwent electron microscopy. MET: Zeiss LEO-906E. Results: MLR formation starts when monocytes transformed in macrophages and increased since 20 h. We observed multivesicular, multilaminar and tubular structures in spatial organization of MIIC along time culture, according to intermediated and late structures described. However in a lymphocyte that does not participate in MLR was observed at 2 h of culture a multilaminar structure like MIIC on plasma membrane. At this time neutrophils were observed whereas at 3h