연제 9

Lectin-binding properties of chicken primordial germ cells during embryonic development

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Abstract

Lectins have great potential as tools to determine the alternation of the distribution of cell surface carbohydrates during cellular development and differentiation. Here, we investigated the presence and distribution of cell surface carbohydrates on chicken primordial germ cells (PGCs) during the migration and gonadal stages using a variety of lectins. A total of six FITC-labelled lectins from several specificity classes were used: Con A (glucose/mannose), WGA (*N*-acetylglucosamine), STA (*N*-acetylglucosamine), DBA (*N*-acetylgalactosamine/galactose), UEA-I (fucose) and PHA-E (oligosaccharide). As a results, PGC-specific binding was observed in STA. PGCs of migration stage (2.5- and 5.5-day embyos) were STA-positive whereas PGCs of 10-day embryonic gonad were not. The results suggest that N-acetylglucosamine residues are present specifically in migrating chicken PGCs and changes during development.
(**Key words** : Lectin, PGCs, STA)

Introduction

The development of PGCs in avian embryos entails a sequence of cellular events, including cell migration to particular sites, proliferation, and morphological differentiation. These events are thought to be mediated by cell-cell interactions or recognitions through cell surface molecules.

It is generally perceived that changes in the completement of cell surface carbohydrate determinant accompany, and may influence many developmental events during early embryogenesis, including cell-cell interactions. Lectins are carbohydrate binding proteins of non-immune origin that interact with specific sugar residues found in glycoconjugates. By using lectins with different and overlapping specificities, it is possible to identify and compare the cellular source of specific types of glycoconjugates. Therefore, lectin histochemistry has been used to determine the distribution of cell surface carbohydrates as alterations in their expressions have been implicated in cellular development and differentiation. In aves, it has been shown that glucose/mannose- and lactose-binding sites are abundant in chick PGC. However, there exist little information avaliable on selectgive carbohydrates present on the surface of the germ cells. In this study, we investigated distribution of cell surface carbohydrates on chicken primordial germ cells (PGCs) during migration and gonadal stages using a variety of lectins. Our results demonstrated that

chicken PGCs are selectively reactive to a specific lectin species.

Materials and Methods

Chicken PGCs were partially purified by using the Ficoll-density-gradient centrifugation from the blood of 2.5-day old embryos and the gonads of 5.5-day and 10-day old embryos. FITC-labelled lectins (20~60 ㎍/㎖) from several specificity classes were used (Table 1). The cells isolated were fixed in 4% paraformaldehyde in 0.1M PBS and spread onto a slide coated with chrom-alum. Lectin-binding reaction was carried out at room temperature for 2 hours in a humidified chamber. Slides were washed several times with PBS and mounted with antifade. Propidium iodide (2 ㎍/㎖) was used for counterstaining. PAS staining was also applied to confirm PGC-specificity of lectins.

Table 1. List of lectins used in this study

Lectin Source	Abbreviation	Binding specificity[1)]
Dolichos biflorus	DBA	GalNAc
Triticum vulgaris (weat germ)	WGA	$(GlcNAc)_2$, NeuNAc
Ulex europaeus I	UEA-I	α-L-Fuc
Canavalia ensiformis (concanavalin A)	ConA	α-D-Man, α-D-Glc
Phaseolus vulagaris agglutinin-E	PHA-E	Oligosaccharide
Sojanum tuberosum	STA	$(GlcNAc)_3$

[1)] GalNAc, N-acetyl-galactosamine; GlcNAc, N-acetyl-glucosamine; NeuNAc, N-acetyl-neuraminic acid; L-Fuc, L-fucose; D-Man, D-mannose; D-Glc, D-glcucose.

Results and Discussion

While lectins, ConA, WGA, DBA, UEA-I and PHA-E, reacted with somatic cells (CEF) and with PGCs of all developmental stages, STA was shown to be specific to chicken PGCs. PGCs of migration stage (2.5- and 5.5-day embyos) were STA-positive while those of 10-day embryonic gonad were not. The results demonstrate that N-acetylglucosamine residues are present specifically in PGCs during their migration and early gonadal settlement periods, and diminished during differentiation.

Therefore, STA can be utilized in differentiating 10-day-old PGCs from migrating and early gonadal PGCs.

References

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