GENETIC EVOLUTION OF HUMAN CORONAVIRUS OC43 IN NEURAL CELL CULTURE

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1. INTRODUCTION

Human coronaviruses (HCoV) are ubiquitous in the environment and are responsible for up to one-third of common colds. HCoV-OC43 possesses a genome that comprises genes encoding various structural and nonstructural proteins. Amongst these proteins, the S protein is biologically very important because it could be involved in determination of viral tropism. Indeed, it could for instance be associated with the capacity of the virus to reach the central nervous system (CNS) and possibly trigger neurological disorders. It could also confer the host species specificity observed with coronaviruses. In past years, we have shown that HCoV-OC43 is neurotropic and neuroinvasive, as it persistently infects neural cell cultures¹ and human brains.² Although we have suggested that OC43 could remain genetically surprisingly stable in the environment,³ it is known that coronaviruses can adapt in cell culture or under selection pressure, for instance related to immune system evasion.

2. MATERIALS AND METHODS

2.1. Viruses, Cell Lines, and Persistent Infections

The ATCC HCoV-OC43 strain (VR-759) was grown on the HRT-18 rectal tumor cell line. Persistent infection were carried out in those HRT-18 cells, as well as in the MO3.13 oligodendrocytic,¹ H4 neuroglial, U-87 MG astrocytic, and TE-671 rhabdomyosarcoma cell lines (ATCC). Other cell lines used for virus susceptibility are described in Table 2. Four infections were performed in the H4 cell line, whereas the HRT-18, MO3.13, and H4 cell lines were acutely infected as controls.

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2.2. Virus Purification

Virus from persistent infections was purified at different passages. Prior to purification, virus was clarified and precipitated with polyethylene glycol (PEG) 8000 (Sigma). Accudenz (Accurate Chemicals) was used to perform gradient purification.

2.3. RT-PCR and Sequencing

Viral RNA was extracted using the GenElute Direct mRNA Miniprep Kit (Sigma) and reverse transcribed with MMuLV reverse transcriptase (Invitrogen). The Expand High-Fidelity Taq polymerase (Roche) was used to perform PCR. Primers specific to the HE, S and N genes were used to amplify target regions. PCR amplicons were purified using the Qiaex II gel extraction kit (Qiagen) prior to sequencing, which was carried out by Bio S&T (Montréal, Québec, Canada).

2.4. Assays for Viral Susceptibility and Modulation of Tropism and Infectivity

Prior to performing assays for modulation of tropism and infectivity, susceptibility of different cell lines to HCoV-OC43, ATCC strain, was determined (Table 2). The same cell lines were then infected with virus isolated from different purifications (HRT-18 P33, P54, P110, and P155; H4 P47 and P90; H4 P56.1, P56.2, P56.3, P116.1, P116.2, and P116.3; TE-671 P38 and P79; U-87 MG P35, and MO3.13 P5, P6, and P22) in order to correlate the observed mutations with a modulation of tropism or infectivity. Supernatants were titrated using an indirect immunoperoxidase assay (IPA), as previously described.

3. RESULTS

Persistent infections of neural cell lines were initially performed to determine whether virus carrying mutations in genes encoding the surface protein S originated as a consequence of viral persistence. The HE protein gene and the nucleocapsid protein gene N were also sequenced in order to determine if these genes contributed to adaptation in cell culture. Viral particles released from persistently infected neural cell lines were isolated and purified by gradient centrifugation, and genomic RNA was sequenced. Results showed various mutations in the S gene but very few in HE and N genes, suggesting that the S gene is responsible for adaptation to the cellular environment, which could be associated with neurotropism, neuroinvasion, and presumably neuropathogenesis (Table 1). Almost every acquired mutation (Table 1) was conserved at subsequent passages, suggesting that they could confer an adaptive advantage and a stable phenotype to the virus. Five mutations were predominant and were found in almost all persistent infections (D24Y, S83T, H183R, Y241H, and N489H). The first four mutations are located in the putative receptor binding site, whereas the fifth one is located within the hypervariable region.

To correlate the observed mutations in the S gene with viral replication and tropism, assays for modulation of tropism and infectivity were performed using cell lines originating from various human tissues as well as from various animal species, for which
### Table 1. Location of S mutations at various passages of persistently infected cells.

| HRT-18 | H4  | H4  | H4  | H4  | TE-671 | U-87 MGMT03.13 |
|--------|-----|-----|-----|-----|--------|-----------------|
| P155   | P90 | P116.1 | P116.2 | P116.3 | P79 | P35 | P22 |
| D30H*  | D24Y | D24Y | D24Y | N25Y | N27Y | D24Y | D115H |
| S83T   | V161V | P35S | P35S | P35S | P35S | P34S | S83T | T148I |
| L85Q   | H183R | S83T | S147Y (D) | S83T | L85R | H183R | Y241H |
| D115H  | V240V | E170K | H183R | Y119H | Y258R | Y241H | M670T |
| T148I  | Y241H | Y183R | Y241H | S147P | A373V | P973S |
| H138Q  | N441K | Y241H | N441K | H183R | R757S | A1090V |
| S258R  | Q541L | A469V | A469D | Y241H | G785D | V1213A |
| S366G  | R570P | R570R | H482Y | N489H | P972L |
| N413T  | N639N | T855I | F853Y | K506T | P973S |
| F420S  | T855I | N880K (I) | L693F | T641S | A978S |
| N489H  | D875H | L893H | A759E | N768T | T1086N |
| K506N  | L893R | S959C | S898S (I) | E896K | D1170A |
| T536N  | A965V | W974L | V980A | S901F |
| Q541L  | T975A | T975P | N1203 (D) | W974L |
| R757H  | I1227T | V980A | I1227T | F982L |
| E896D  | T1245I | S1093S | V986I |
| C897G  | G1169G | G1169D |
| E933G  | M1222K | E1236A |
| F982L  | D1232Y |
| S1192R | P1249L |
| T1225J | I1304I |
| P1228S |

* D, deletion; I, insertion. ** Passages (and purification numbers) are indicated below the cell line.

Susceptibility to HCoV-OC43 infection was previously determined (Table 2). These analyses revealed that mutations found throughout the S gene could affect the latter viral properties in certain cell lines. Amongst the virus variants obtained following persistent infections and virus purifications, five showed extended cellular tropism and increased replication titers in vitro: U87-MG P35, H4 P47, H4 P56.3, H4 P116.1, and H4 P116.2 (data not shown). Furthermore, some variants isolated from persistent infections were more virulent in mice and could form plaques, in opposition to the ATCC HCoV-OC43 reference strain VR759 (data not shown).

### 4. DISCUSSION

We have identified several mutations in the S gene of the HCoV-OC43 genome following persistent infections in different cell lines. These mutations will help us to further characterize viral adaptation during persistence and to understand mechanisms that are implicated in viral tropism and infectivity. Future studies will be carried out using an infectious cDNA clone of the OC43 strain assembled in a BAC vector.7,8 The construction of this clone was performed in collaboration with F. Almazán and L. Enjuanes and will provide an invaluable tool to further understanding the underlying mechanisms for viral replication and tropism. In combination with the experiments described above, the clone will be useful in elucidating the molecular basis of human coronavirus neuropathogenesis.
Table 2. Susceptibility of various cell lines to the HCoV-OC43 ATCC strain.

| Cell line | Origin | Tissue Type | IPA 1 | Susceptibility 2 |
|-----------|--------|-------------|-------|------------------|
| HeLa      | Human  | Uterus Epithelial | 3.75  | Low              |
| MT4       | Human  | Bone marrow T lymphocyte | 2.0   | No               |
| U937      | Human  | Bone marrow Monocyte | ≤0.5  | No               |
| Jurkat E6.1 | Human  | Bone marrow T lymphocyte | ≤1.5  | No               |
| Raji      | Human  | Bone marrow B lymphocyte | 4.5   | Yes              |
| HL-60     | Human  | Bone marrow Monocyte | 3.25  | Low              |
| WI-38     | Human  | Lung Fibroblast | 4.0   | Yes              |
| L132      | Human  | Lung Epithelial | 3.25  | Low              |
| Caki-2    | Human  | Kidney Epithelial | ≤1.5  | No               |
| SW 156    | Human  | Kidney Epithelial | ≤1.5  | No               |
| NCI-N87   | Human  | Stomach Epithelial | ≤1.75 | No               |
| Arpe-19   | Human  | Eye Epithelial | ≤1.5  | No               |
| FHs 74 Int | Human  | Intestine Epithelial | 3.5   | Low              |
| TK6       | Human  | Spleen T lymphocyte | ≤1.75 | No               |
| 17 Cl-1   | Mouse  | Embryo Fibroblast | ≤1.75 | No               |
| L929      | Mouse  | Subcutaneous Fibroblast | ≤1.5 | No               |
| N-11      | Mouse  | Brain Microglial | ≤1.5  | No               |
| DBT       | Mouse  | Brain Gial | ≤2.25 | No               |
| J774 A.1  | Mouse  | Bone marrow Macrophage | 4.5   | Yes              |
| A20       | Mouse  | Bone marrow B lymphocyte | 3.25  | Low              |
| S END.1   | Mouse  | Skin Endothelial | ≤1.5  | No               |
| Cos-7     | Monkey | Kidney Fibroblast | ≤1.5  | No               |
| Vero      | Monkey | Kidney Epithelial | ≤1.75 | No               |
| Vero E.6  | Monkey | Kidney Epithelial | ≤1.5  | No               |
| B104      | Rat    | Brain Fibroblast | 3.25  | Low              |
| BHK-21    | Hamster| Kidney Fibroblast | 4.0   | Yes              |

1 Indirect immunoperoxidase assay (infectious titers in TCID₅₀/mL).
2 Titers from 0 to 3, not susceptible; titers over 3 and under 4, low susceptibility; titers of 4 and over, susceptible.

5. REFERENCES

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