Primitive cells are most sensitive to human fetal DNA

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Introduction

A trio of recent publications in the journal NEURON reports the presence of hundreds of diverse de novo gene mutations indicating that autism spectrum disorder (ASD) may be a disease of genomic instability, with a significant environmental component. Altered double strand break (DSB) formation and repair pathways may be a commonality among the diverse genetic mutations that have been documented in ASD. US birth year change points in AD are apparent in 1980, 1988 and 1996, coinciding with the switch to or introduction of childhood vaccines contaminated with human endogenous retrovirus K (HERVK) and human fetal DNA fragments (6). We hypothesize that the HERVK and human fetal DNA contaminants could contribute to the genomic instability of ASD as demonstrated by de novo mutations.

Cell free DNA can be taken up by healthy cells via receptor mediated uptake or may spontaneously penetrate cell membranes that have altered permeability, for instance, during inflammatory reactions. Nuclear uptake of cell free DNA fragments is thought to provide a source for maintenance of DNA integrity during rescue of collapsed replication forks or base lesion repair. Spontaneous extracellular DNA uptake has also been exploited for gene therapy as well as for cellular gene correction (2,4,5,7,8, and 9). While free DNA uptake has been used advantageously, the process has also been associated with generation of mutations and chromosomal aberrations (3).

Surprisingly primitive cells, such as stem cells and embryonal cells take up foreign human DNA most efficiently. Liu *et. al.* demonstrated short fragment DNA integration in CD34(+) stem cells and more primitive Lin(-)CD38(-) stem cells; DNA integration efficiency was 1.7% (10) (Fig1). Chin *et. al.* also observed an excellent efficiency of small fragment DNA integration in primary human CD34+ stem cells (11). Similarly, McNeer *et. al.* delivered DNA fragments using nanoparticles, and demonstrated 0.01 to 0.04% genomic integration in human CD34(+) stem cells (12) (Fig2). These studies suggest that foreign DNA uptake and integration are more likely to happen in primitive cells. This increases risks of mutagenesis in various organs through dissemination of stem cells carrying foreign DNA in the host body.

Vaccines manufactured using human fetal cells contain residual DNA fragments (50-500 bp) (Table 1). It is possible that these contaminating fragments could be incorporated into a child's genome and disrupt normal gene function, leading to autistic phenotypes. In this study we demonstrate foreign DNA uptake in human cells and genomic integration by incubating the cells with Cy3-labeled human Cot1 (placental) DNA fragments which represents contaminating residual human fetal DNA in vaccines.

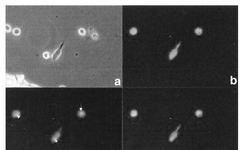


Fig1. The arrows in Picture.C indicateTexas Red conjugated short fragment of DNA maintained and concentrated in Lin(-)CD38(-1) cells . Liu *et. al.*

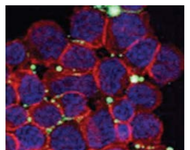


Fig 2. Nanoparticles (green) carrying short fragment of DNA taken up by CD34(+) cells. McNeer *et.al.*

Table 1. Levels of residual human single stranded DNA (Oligreen assay) and human double stranded DNA (Picogreen assay) in Rubella vaccine (Meruvaxll) and Hepatitis A vaccine (HAVRIX).

Vaccine name	Single Stranded DNA (ng/vial)	Double Stranded DNA (ng/vial)	Length (bps)
Meruvax II (Rubella)	142.05	35.00	240
HAVRIX (Hepatitis A)	276.00	35.74	Not measurable

Materials and Methods

Human Cot1 DNA (Invitrogen) was labeled with Mirus *Label* IT Cy^{TM}3 *Labeling Kit* (Mirus).

For spontaneous DA uptake, U937 cells (human lymphocytes) and HL-60 cells (human myeloblasts) were incubated with 750µg of Cy3 labeled human Cot1 DNA per 1.0x10^7 cells for 24 hours and 48 hours at 37°C. Loosely adherent NCCIT (Pluripotent Enbryonal Carcinoma) and HFF1 (Human Foreskin Fibroblast 1), BE (2)-C (neuroblastoma) cells, M059K (Glioblastoma-Double Stranded Break repair proficient) and M059J (Glioblastoma-Double Stranded Break repair deficient) cells were incubated with 500ng Cy3 labeled human Cot1 DNA per 3x10^4 cells for 24 hours and 48 hours at 37°C. Cellular and nuclear DNA uptake of U937 cells and NCCIT cells was analyzed under fluorescent microscope. Genomic DNA of U937 cells was purified by ethanol precipitation removing short fragment of host nucleic acids including unincorporated Cy3 labeled human Cot1 DNA. The amount of Cy3 labeled human Cot1 DNA incorporated into chromosomes of each cell line was calculated with relative fluorescent unit (RFU) measured by a fluorimeter

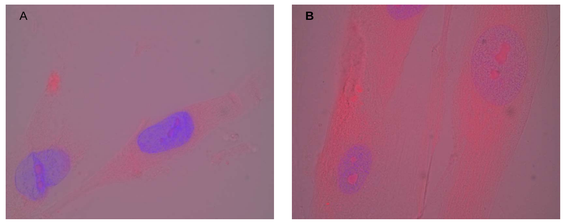
To model inflammation, all adherent cell lines were activated with lipopolysaccharide (LPS). Saponin permeabilization was tested for HFF1 cells. Cancer drug, colcemid was used for U937. Three concentrations of LPS, 1ng/10^{4}cells, 10ng/10^{4}cells, and 100ng/10^{4}cells were tested in the wells of each cell line previously mentioned. Cells were incubated with Cy3 labeled human Cot1 DNA and LPS at 37°C for 24 hours and 48 hours. As well as cells incubated without LPS, these cells were also stained with Hoechst before cellular and nuclear DNA uptake was analyzed under fluorescent microscope. HFF1 cells were incubated with 0.02% saponin, 300ng/ml DAPI, and 500ng Cy3 labeled human Cot 1 DNA for 24 hours, 48 hours, and 72 hours. Cells were viewed under fluorescent microscope as well. 1x10^6 U937 cells were incubated with 0.04µg/ml and 0.05µg/ml colcemid for 24 hours and 48 hours, and cellular and nuclear DNA uptake was analyzed under fluorescent microscope.

Results: (Table 2): Spontaneous cellular and nuclear DNA uptake was evident in HFF1 (Fig A and B), U937 (Fig C, D, E, and F), HL-60 (Fig G and H), and NCCIT (I and J). M059K was not measurable because of high auto fluorescence of the cells. No Cy3 signal was observed in HL-60. With inflammation cellular and nuclear DNA uptake was observed in HFF1 (Fig K and L), and U937(Fig M and N). Cellular DNA uptake with inflammation was observed in NCCIT (Fig O and P), M059J (Fig Q and R) and BE(2)-C (S and T).

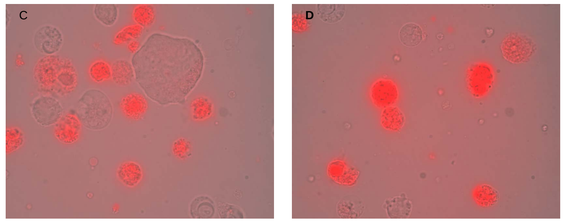
The amount of labeled Cy3 human Cot1 DNA incorporation in U937 genomic DNA was 0.0111 +/- 0.0034pg (n=12) per cell in 24 hours, which was approximately 0.167% of total U937 genomic DNA. DNA incorporation in NCCIT cells was 0.0026pg/cell in 24 hours and 0.04pg/cell in 48 hours which is 0.6% of total NCCIT genomic DNA.

Results

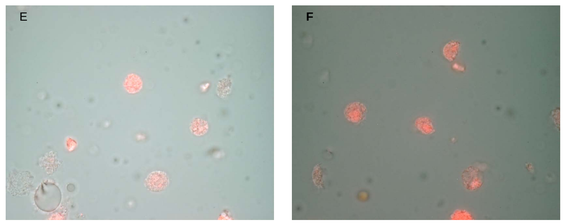
Spontaneous Cellular & nuclear DNA up take



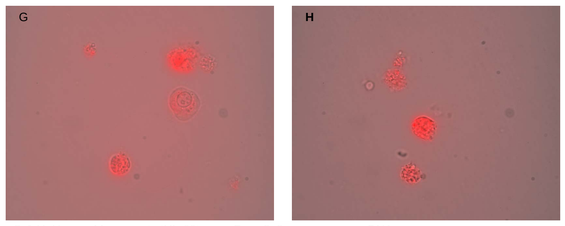
A & B: Human Foreskiin Fibroblast (HFF1) Red: Cy3 labeled Human DNA , Blue: Hoechst labeled nucleus



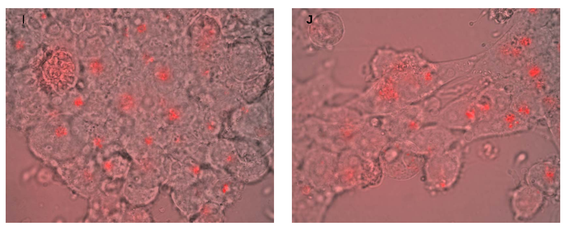
C & D: Human Lymphocytes (U-937) Red: Cy3 labeled human DNA



E & F: Human Lymphocytes (U-937) Nucleus Red: Cy3 labeled human DNA

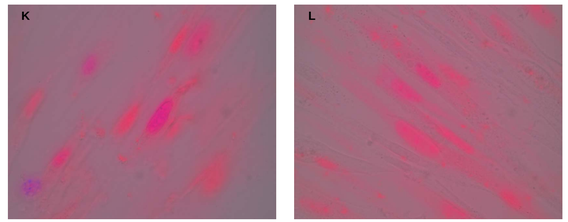


G & H: Human Myeloblasts (HL-60) Red: Cy3 labeled human DNA

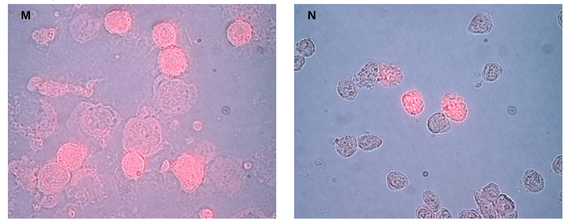


I & J: Human Pluripotent Enbryonal Carcinoma (NCCIT) Red: Cy3 labeled human DNA

Cellular & nuclear DNA up take by inflammation

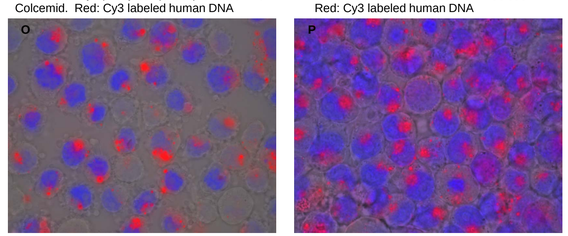


K & L : Human Foreskin Fibroblast (HFF1) treated with saponin
Red: Cy3 labeled human DNA, Blue: DAPI labeled nucleus

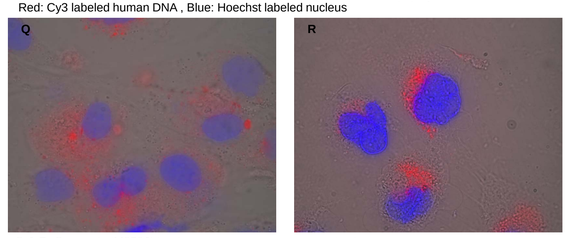


M: Human Lymphocytes (U-937) treated with Colcemid. Red: Cy3 labeled human DNA

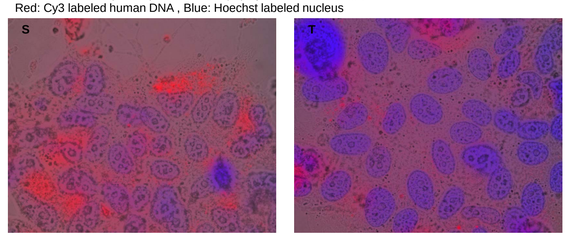
N: U-937 treated with Colcemid . Nucleus Red: Cy3 labeled human DNA



O & P: Human Pluripotent Embryonal Carcinoma (NCCIT) treated with lipopolysaccharide
Red: Cy3 labeled human DNA , Blue: Hoechst labeled nucleus



Q & R: Glioblasts (M059J) treated with lipopolysaccharide activation.
Red: Cy3 labeled human DNA , Blue: Hoechst labeled nucleus



S & T: BE(2)-C Neuroblasts treated with lipopolysaccharide activation
Red: Cy3 labeled human DNA , Blue: Hoechst labeled nucleus

Table 2: DNA uptake in Various Cell lines

	Spontaneous Cellular uptake	Spontaneous Nuclear uptake	Incorporation in Genomic DNA	Cellular /Nuclear Uptake with LPS or saponin
HFF1	Yes	Yes	Not Done	Increase/Increase
NCCIT	Yes	Yes (variable)	0.0026pg per cell 24 hrs 0.04pg per cell 48 hrs	Same/Same
BE(2)-C	Yes	No	Not Done	No/No
M059K	No	No	No	No/No
M059J	No	No	Not Done	Yes/No
U937	Yes	Yes	0.011 +/- 0.003pg per cell 24 hrs	Same/Same
HL60	Yes	Yes	No	No

Discussion

Our measured genomic incorporation (0.003 to 0.04 pgs) of 0.2% - 0.6% (0.003 to 0.04 pgs) of the whole genome in 24 to 48 hours seems high at first glance. However, our numbers are consistent with previous reports showing that exogenous DNA replaced up to 1% of the whole genome within 30 minutes (7). Although HL-60 cells did not spontaneously take up exogenous DNA in our experiments, the cell line has been used in the past as a model for spontaneous DNA uptake (8).

Cellular and nuclear DNA uptake in human foreskin fibroblast (HFF1) cells, in U937 cells and in NCCIT cells suggests that neonatal and embryonal cells, which are relatively primitive cells, are more susceptible to foreign DNA uptake than more mature cell types. These results indicate the need for further study of DNA incorporation from exogenous sources to compare the susceptibility of infants and toddlers versus teens and adults.

Increased DNA uptake after LPS activation suggests that systemic inflammation or immune responses could increase susceptibility for exogenous DNA uptake. Human diploid cell produced vaccines are contaminated by exogenous DNA fragments and a retrovirus, and vaccines elicit systemic inflammation and immune activation. Our future research goals are to localize the sites of DNA integration, to demonstrate phenotype changes caused by foreign DNA integration in factor dependent cell lines, and to determine the biological and/or pathological activities of Human Endogenous Retrovirus K (HERVK) fragments in vaccines.

Conclusion

Foreign human DNA is spontaneously taken up by stressed human cells and even by normal healthy human cells. The foreign human DNA can readily insert into the cell's genome. Primitive human cells, like stem cells, take up and integrate foreign human DNA more efficiently than mature human cells. A stem cell that takes up and integrates foreign DNA could transfer this mutation throughout the body and cause disease. Residual foreign human DNA and retroviral DNA contaminants in vaccines could contribute to various mutations and diseases in infants and toddlers via DNA uptake and incorporation into their vulnerable cells and genome. Hence, vaccines should be produced ethically so that they are not contaminated with human fetal DNA contaminations or reactivated retroviruses and so that the safety of our children can be ensured.

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