A scientific **conference** to celebrate the **50th** anniversary of the International Agency for Research on Cancer(IARC 50th Anniversary Conference)

Title:

Exposure of hepatocellular carcinoma cells to low-level As_2O_3 causes an extra toxicity pathway via L1 retrotransposition induction

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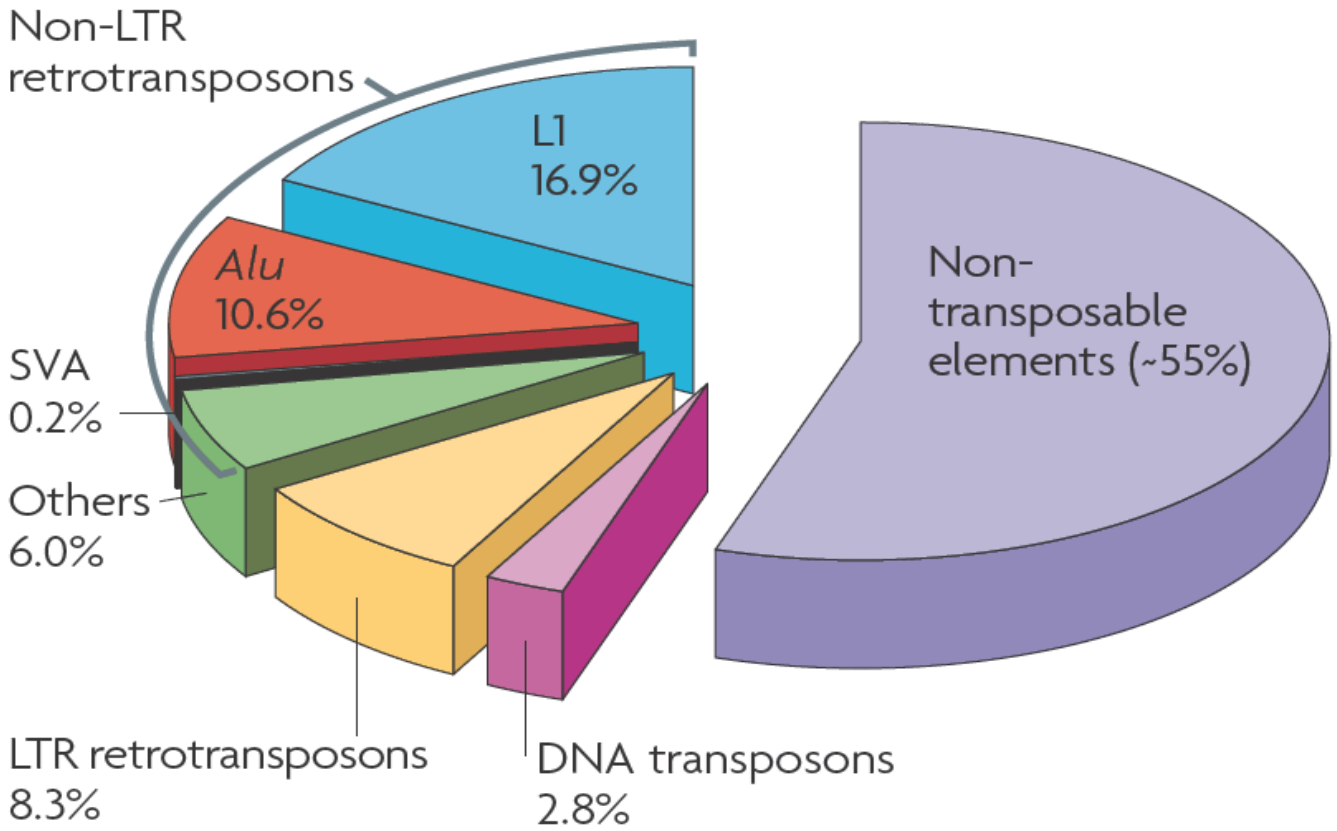
Transposable Elements (TEs)

- ✓ Repetitive sequences account for about 40–45% of mammalian genomes
- ✓ The majority of these repetitive elements are transposable elements (TEs)
- ✓ a group of repetitive sequences that bring positive, negative, as well as neutral effects to the host organism



Human long interspersed element -1 (LINE1/ L1)

- Non LTR retrotransposons
- More than 17% of human genome
- More than 99% of L1s are inactive
- because of point mutation, truncations, other rearrangements
- 80-100 retrotransposition-competent L1

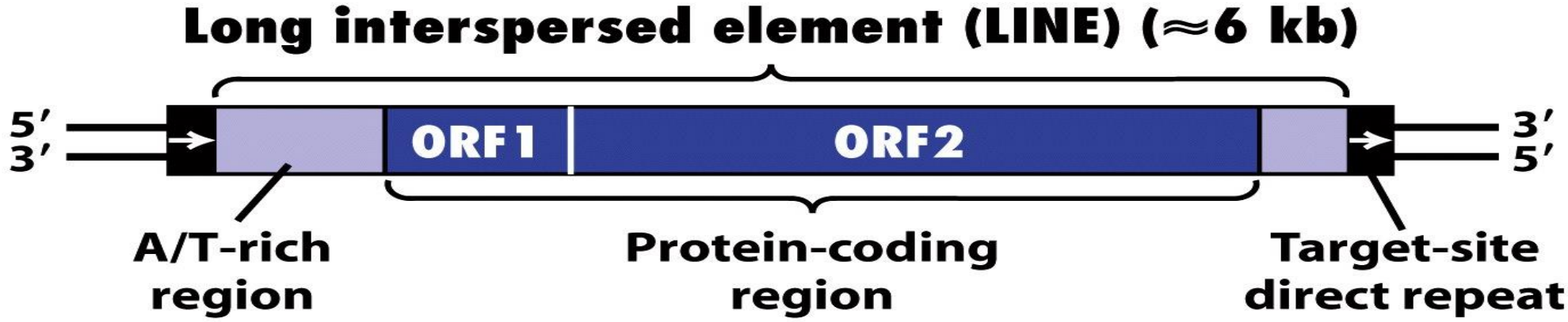


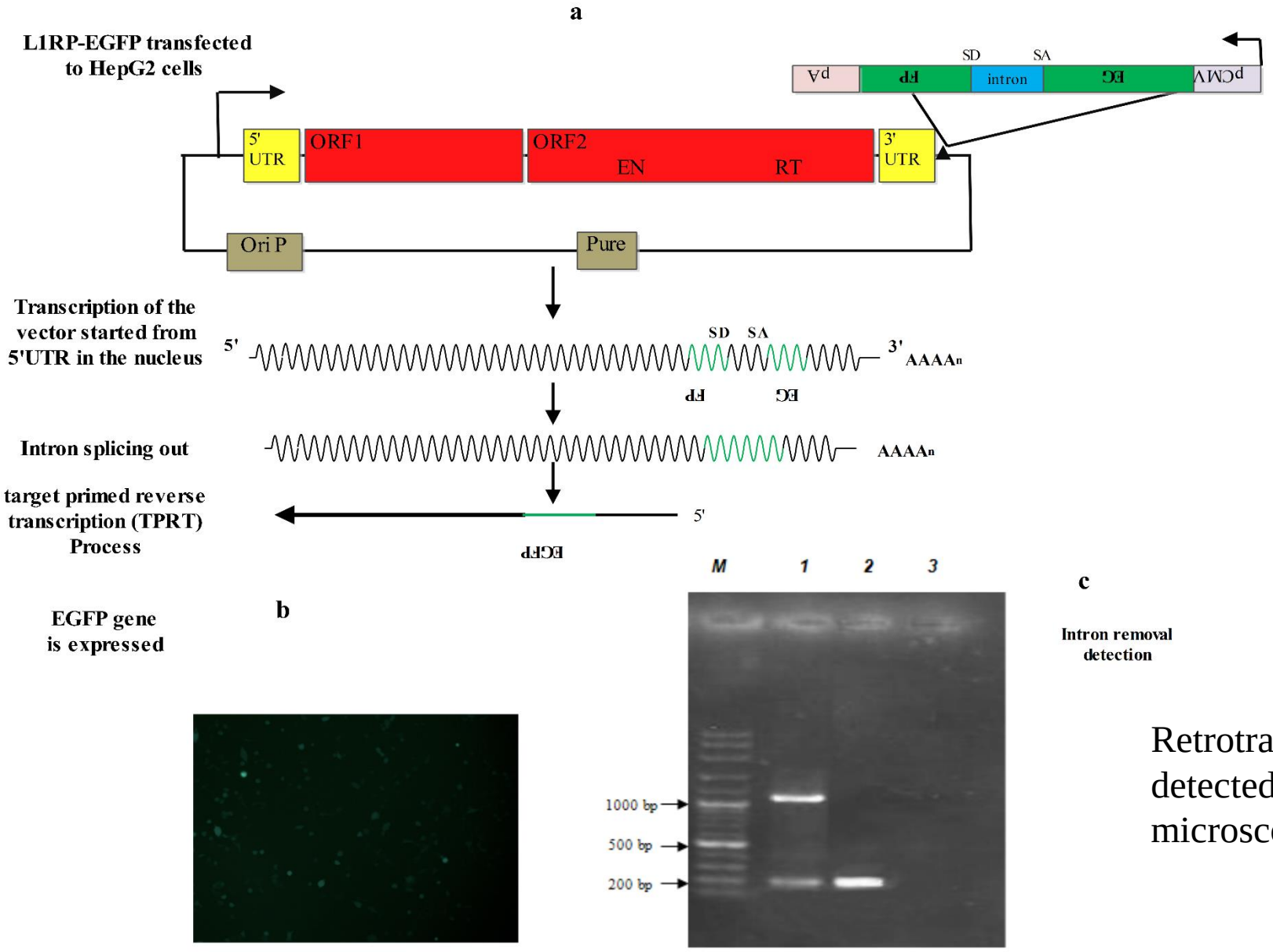
Figure 6-16
Molecular Cell Biology, Sixth Edition

Potential impact upon the human genome

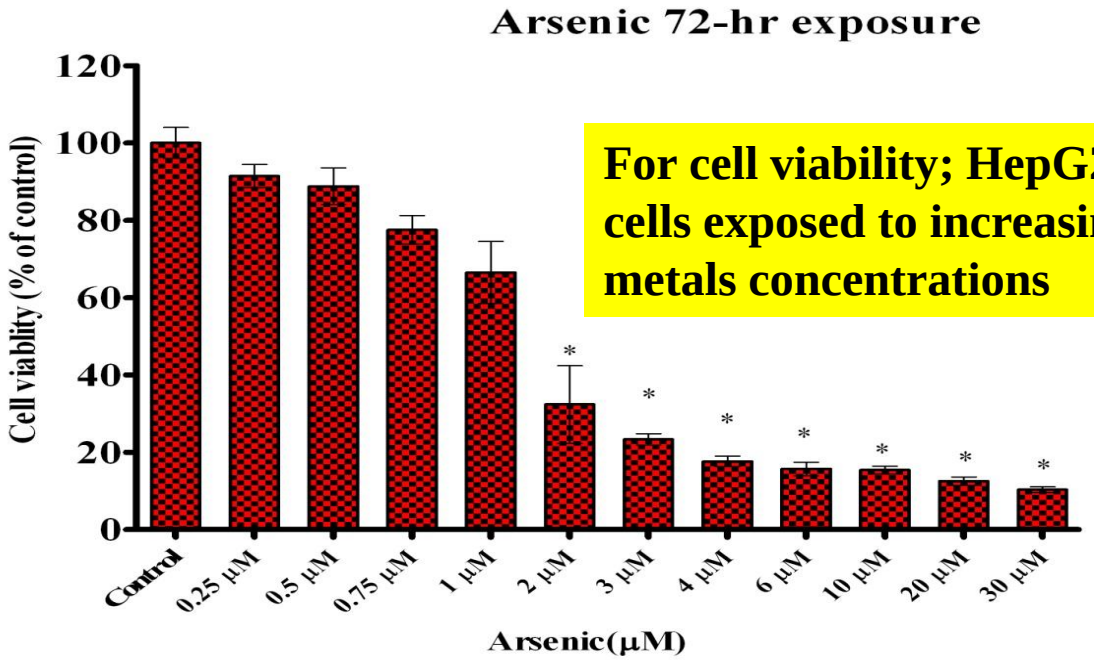
- ✓ Disrupt coding sequences of genes.
- ✓ Damage regulatory sequences.
- ✓ Mediate chromosomal rearrangements resulting in genomic instability
- ✓ L1 activation: colorectal, hepatocellular carcinoma, lung, ovarian, breast and prostate tumors
- ✓ Environmental stressors can affect L1 retrotransposition: oxidative stress, gamma irradiation and X- rays, benzo[a]pyrene (B[a]P), organochloride pesticides, food-borne carcinogens, and some heavy metals

The aim of this study was to evaluate the possible effect of low-level As_2O_3 on L1 retrotransposition alteration in human hepatocellular carcinoma cells (HepG2).

A schematic construct of LINE1 retrotransposition in pCEP4 vector backbone and protocols used to make L1 tracking

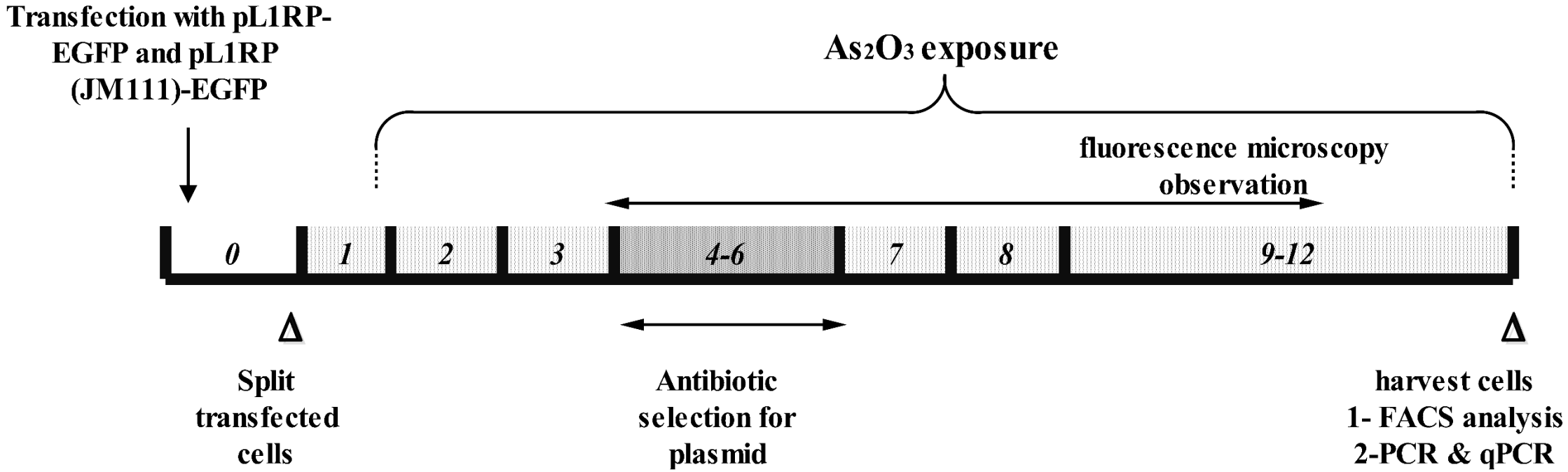


Retrotransposition events was detected by fluorescence microscopy and PCR



For cell viability; HepG2 cells exposed to increasing metals concentrations

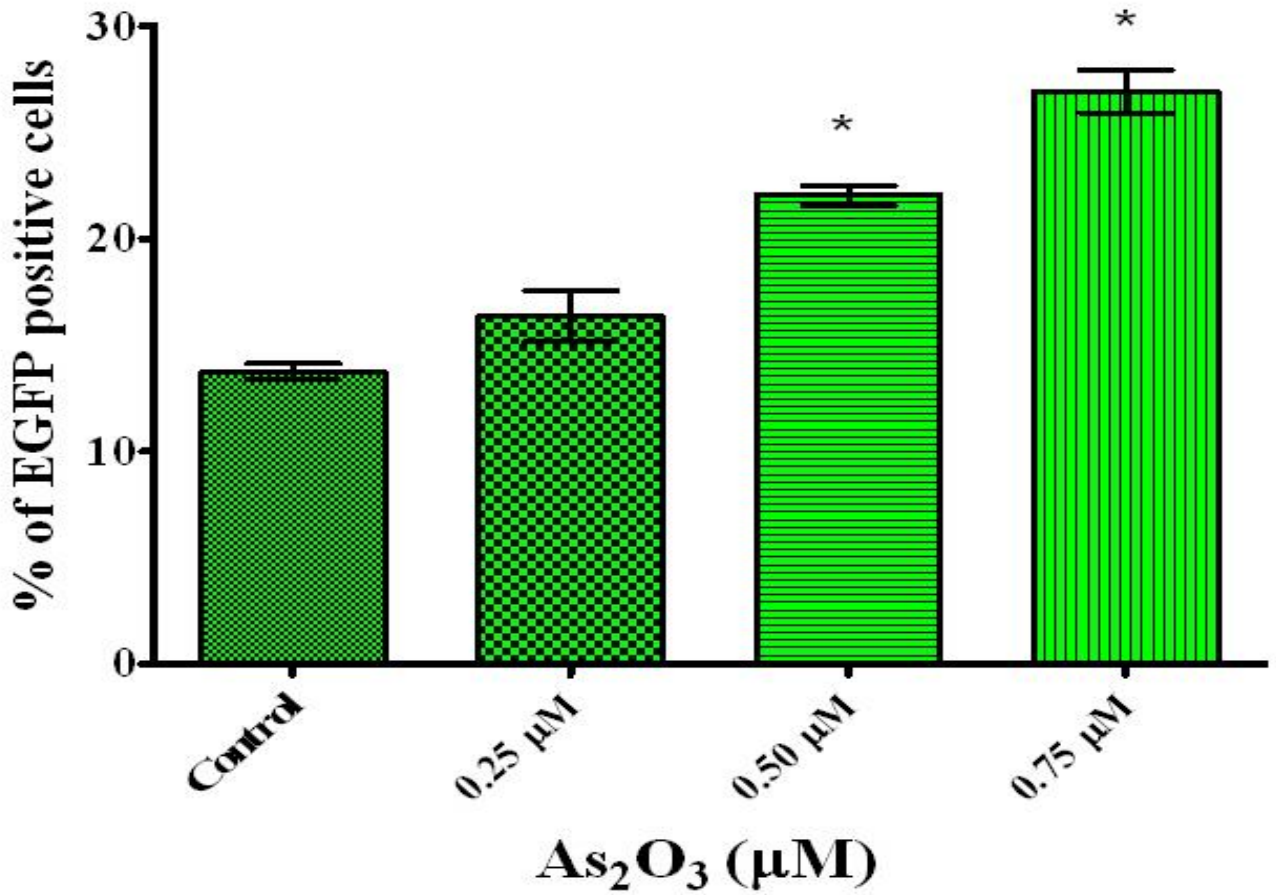
Schematic protocols and timeline of experiment



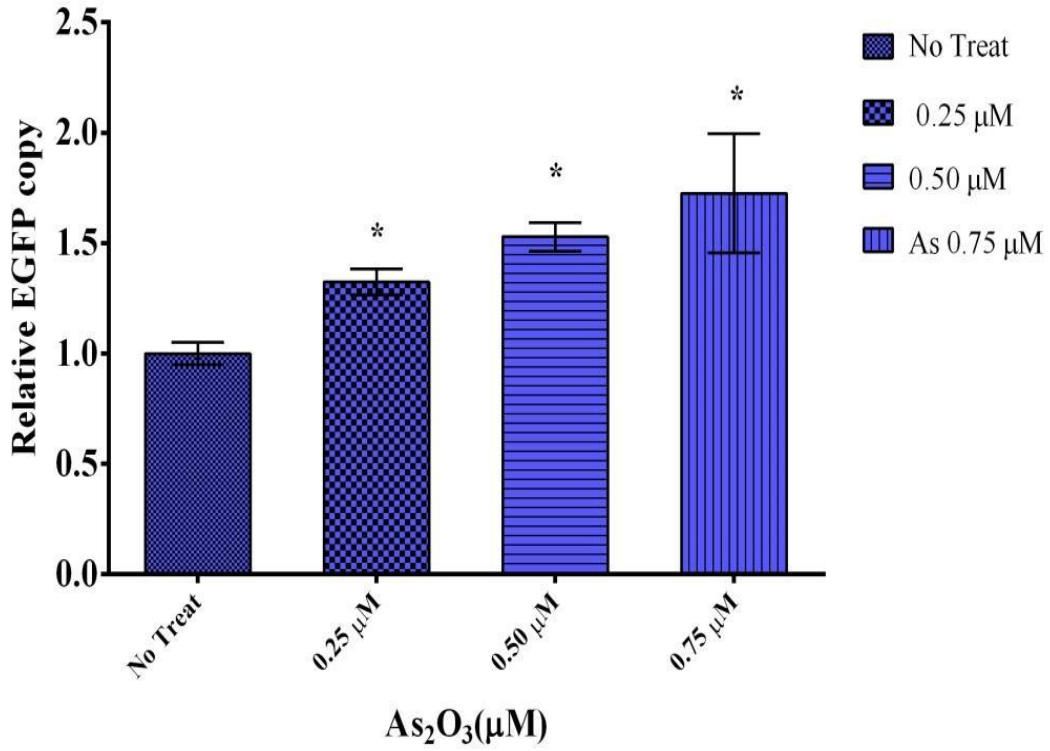
FACS Results

Net frequencies of EGFP-positive cells have been indicated as the mean values ± standard error of three experiments

The effect of As_2O_3 on EGFP copy number, representing L1 retrotransposition on hepG2 cells



qPCR Results



As_2O_3 (µM)	Inserted L1 copies in HepG2 cells genome After 12 days post transfection	Given Genome (Cell)	L1 Number/5000 genome
0	98	1986	**247**
0.25	35	680	**257**
0.50	70	1200	**291**
0.75	40	700	**285**

Absolute quantitation: initial estimations suggested approximately more than 200 insertions in 5000 genomes.

EGFP copy number analysis after treatment of transfected HepG2 cells with As by relative qPCR analysis

Conclusion and Suggestion

- **We first showed EGFP tagged L1 has strong movement in hepatocellular carcinoma cells and Arsenic increased L1 retrotransposition events.**
  - Such events can affect the gene expression profile of vital genes and make cells susceptible to cancer development over time. We believe that the data presented here provides a new paradigm for drug safety assessment

- It is worth pointing out that *Alu* and *SVA* elements, hijacks L1 transcriptional machinery for transposition, However, the depth of retrotransposition consequences by $\mathbf{As_2O_3}$ exposure may be at a much higher rate

- **Screening** for retro-transposon associated biomarkers such as induction of **ORF1 protein in serum** arising from cumulative past metal exposure can be effective and feasible for early detection of most cancers.

Acknowledgement

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Thanks for your attention