

**Application of real-time monitoring of glutathione in living cells
using FreSHtracer, a reversible glutathione sensor, for
development of cell therapy**

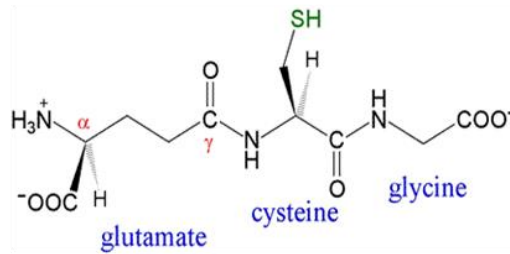
Eui Man Jeong

**Department of Biochemistry and Molecular Biology,
Seoul National University College of Medicine**

Glutathione (GSH): Major ROS Buffering System

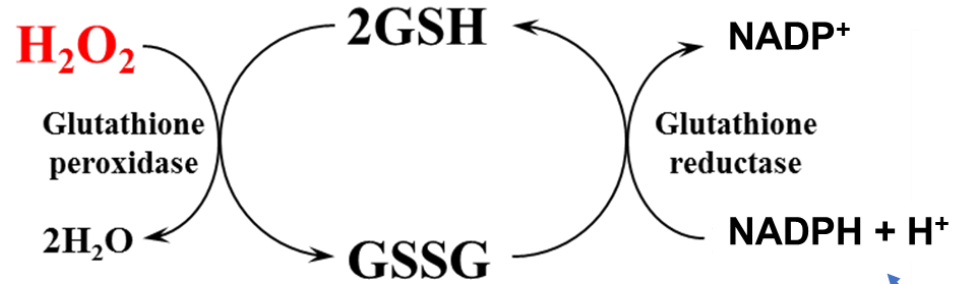
Glutathione (GSH)

Abundance



$[GSH]_i = 1\sim 10\text{mM}$

GSH plays a major role in ROS removal



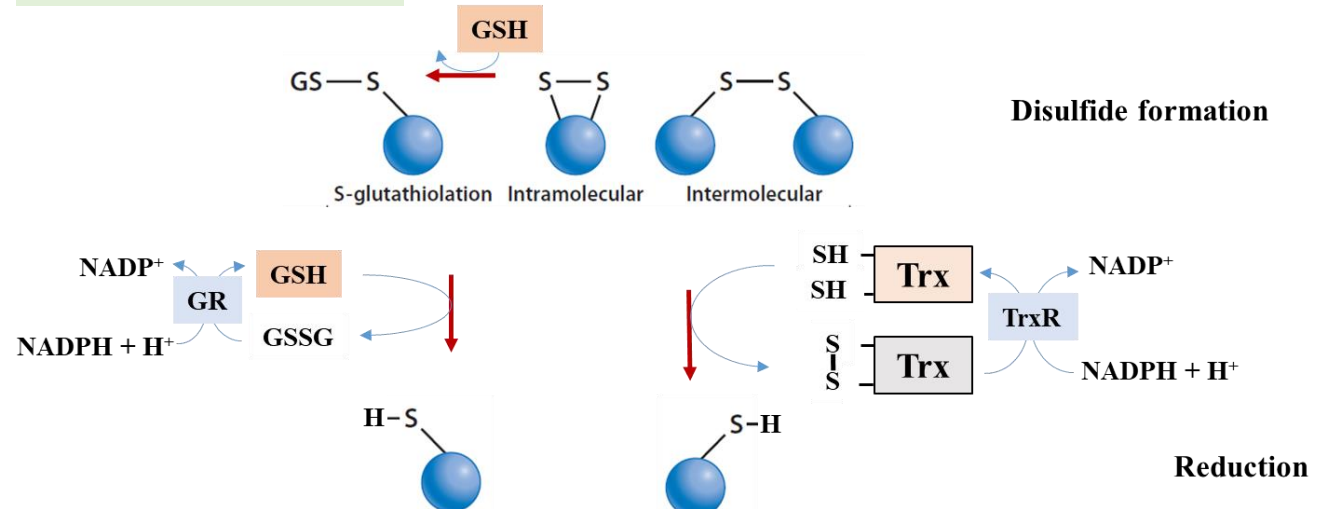
$[GSH]_i \approx$ intracellular Redox status

1. Pentose P Pathway
2. Malic enzyme
3. Isocitrate DH

Reactivity of GSH with ROS

| Oxidants | Vit C | Vit E | GSH |
|---------------|-------|-------|------|
| H_2O_2 | - | - | + |
| Superoxide | + | - | + |
| Peroxynitrite | + | + | ++ |
| HOCl/HOBr | +++ | + | ++++ |
| Haloamines | + | + | ++ |
| Oxyradicals | ++++ | +++ | ++ |

Redox signaling

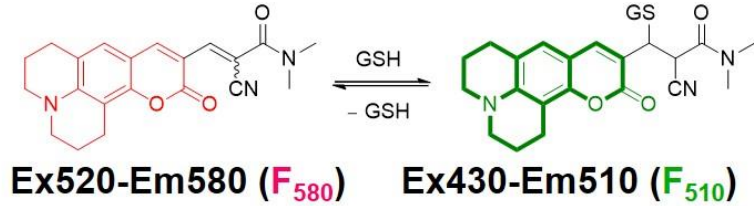


Disulfide formation

Reduction

FreSHtracer : The fluorescent dye to safely measure the level of Glutathione(GSH) in living cells

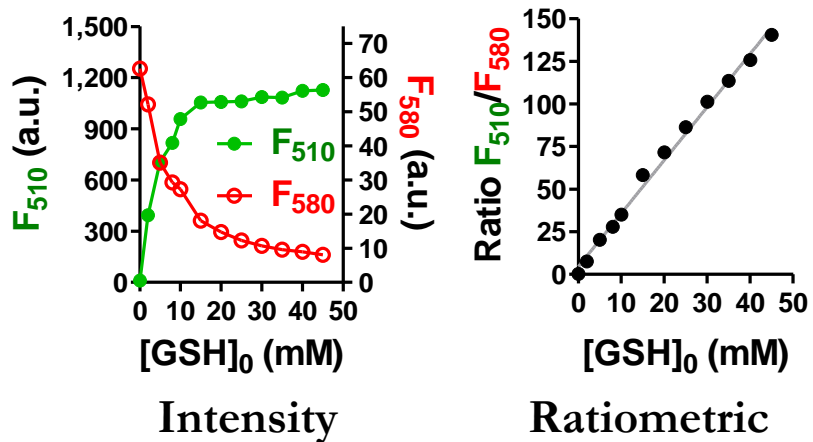
Spectral shift



FreSHtracer

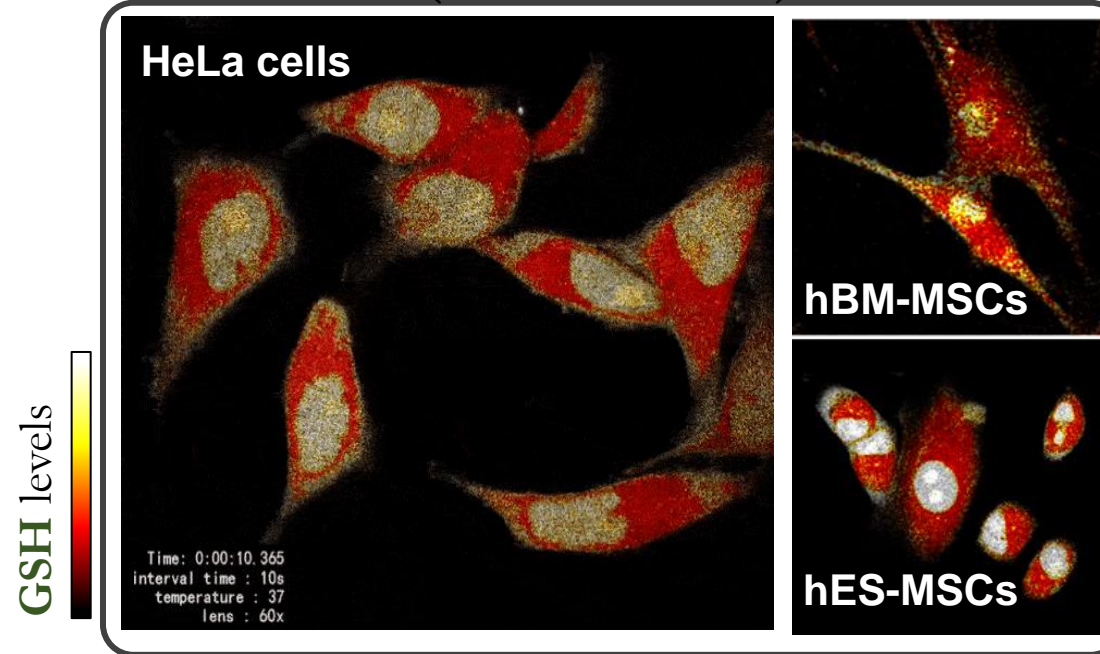
(Fluorescent real-time thiol tracer)

Concentration-dependent fluorescence



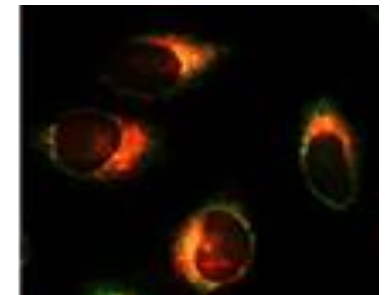
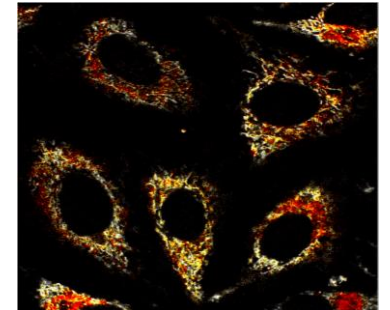
Non-intrusive

FreSHtracer (whole cell area)

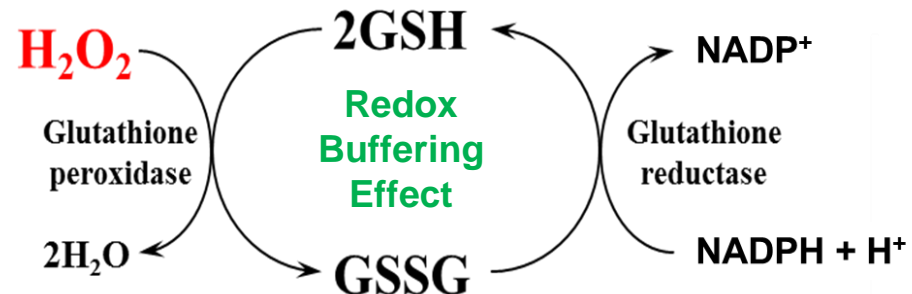


Variable

MitoFreSHtracer

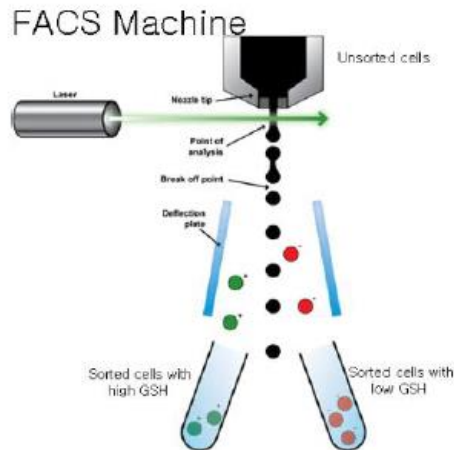
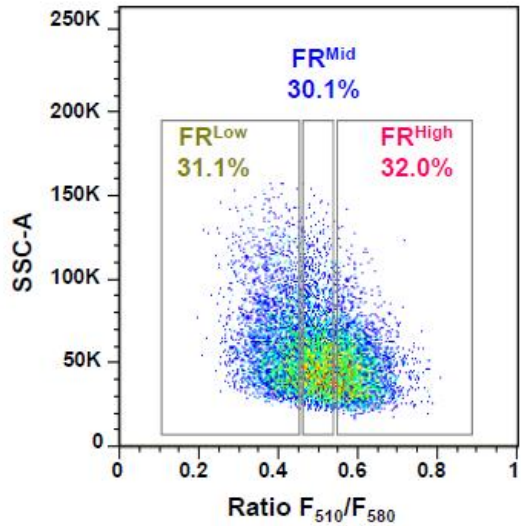


GolgiFreSHtracer



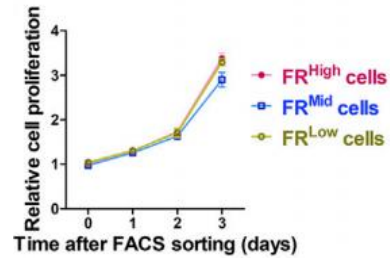
High GSH levels are required for maintaining functional potency of stem cells

Stem cell sorting by GSH level using FreSHtracer

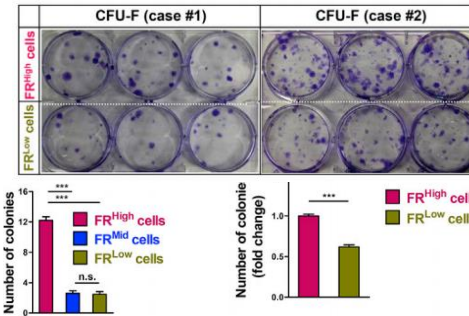


High GSH levels are required for Stem cell function (hES-MS-C)

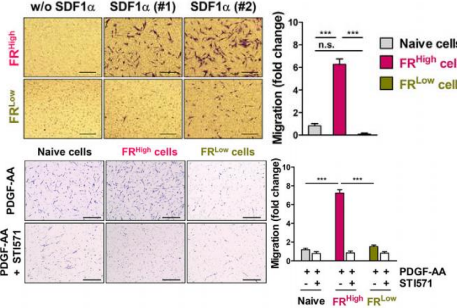
Proliferation



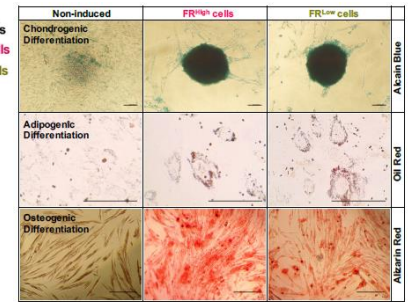
Colony formation



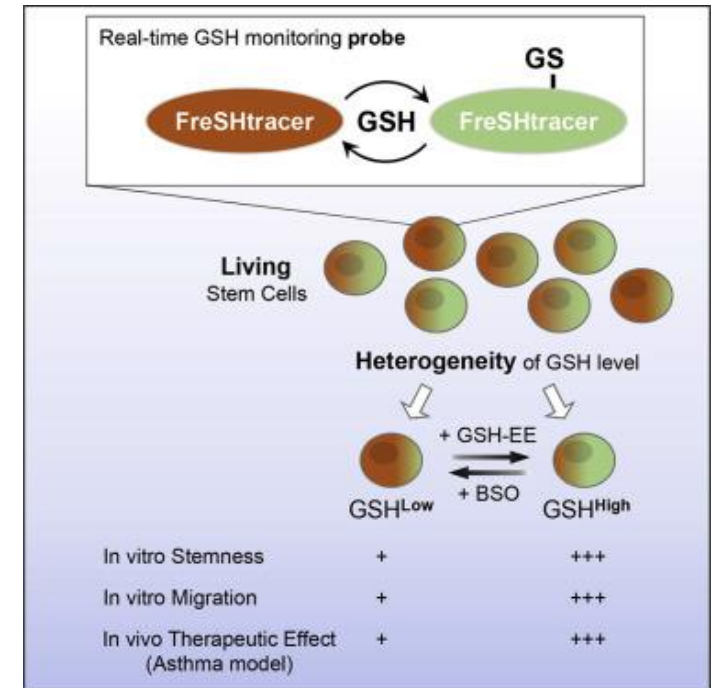
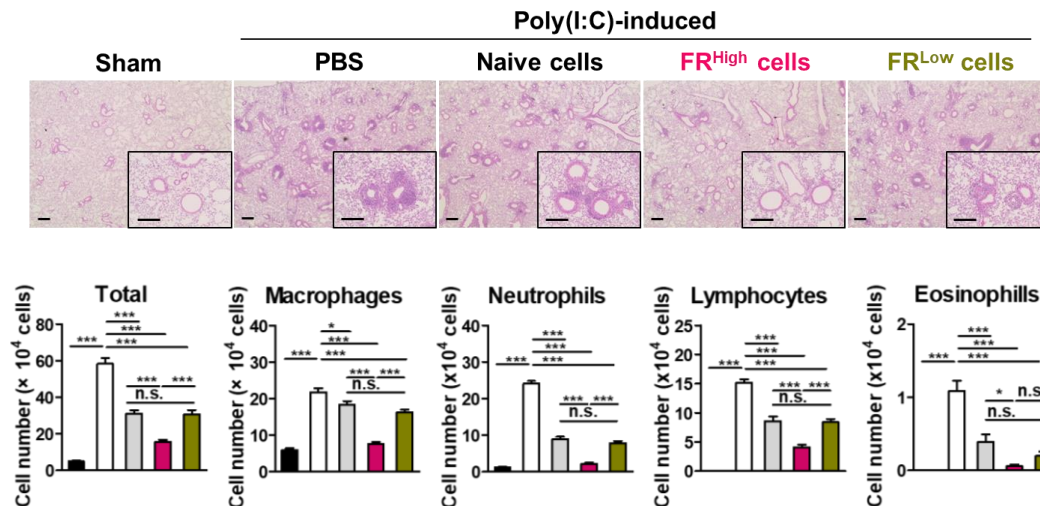
Migration



Differentiation



GSH^{High} Stem cells show Higher Therapeutic Effectiveness in a mouse model of Asthma



Role of Reactive Oxygen Species (ROS) in Stem Cells

ROS: critical factor for **Stemness**

Stem cells are

**located in
Hypoxic Niche**

**dependent on
Glycolysis**

