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RNA: The Key to Improving Platelet Transfusions?

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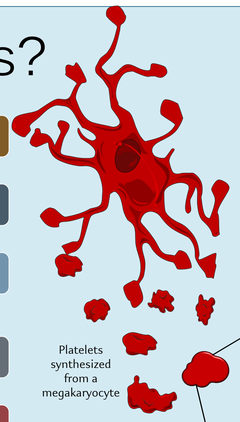
What are platelets?



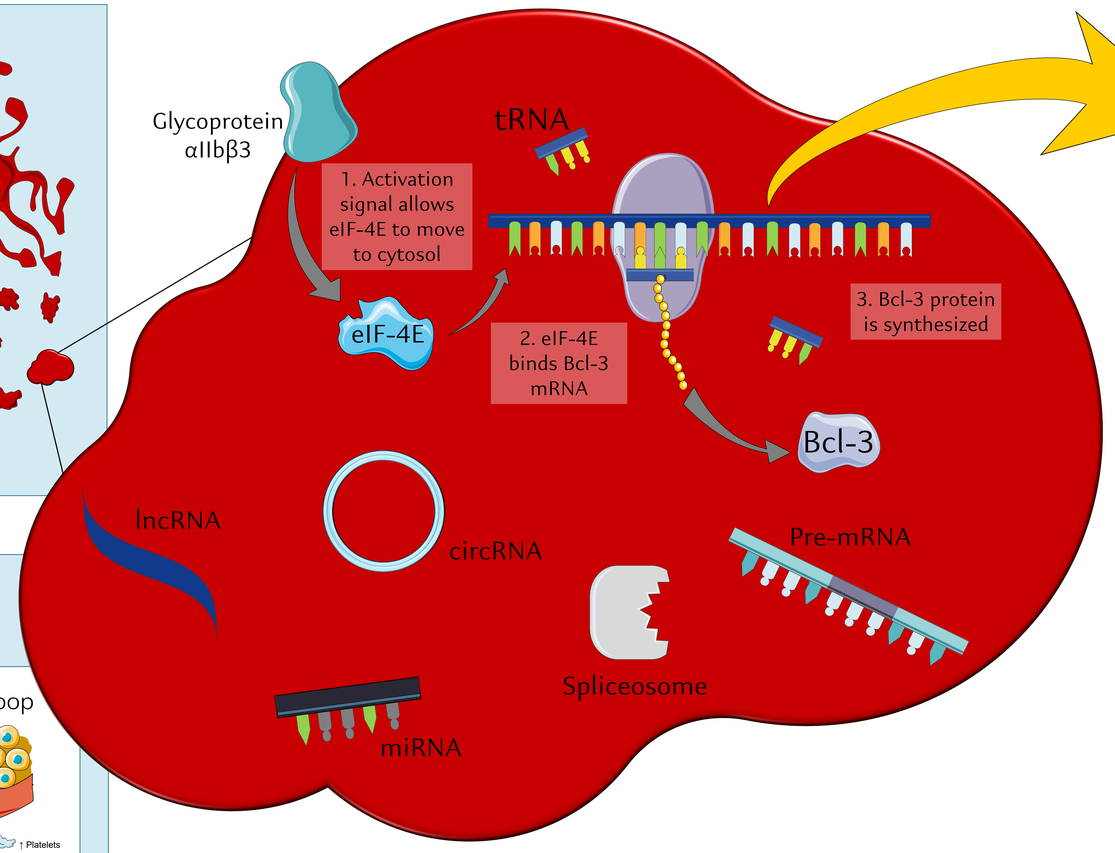
Small anucleate cells in the bloodstream
- Synthesized by megakaryocytes

Have a circulation lifespan of ~7-10 days
- Ideal time to deliver therapies (e.g. drug delivery)

Foundation for initial stages of clotting
- 1st responders that enable a clot to form through a series of reactions

Important in immune response
- At the site of injury recruit cytokines, and promote

Natural carriers
- Take up many different foreign particles



Schematic of platelet protein synthesis pathway for the B-cell lymphoma-3 (Bcl-3) protein, which is involved in clot clearance (top mechanism). This shows the role of RNA control in protein synthesis, instead of DNA. The platelet must receive an activation signal and then the eukaryotic initiation factor-4E (eIF-4E) can move into the cytosol. Once in the cytosol, eIF-4E can bind to Bcl-3 mRNA and enables the ribosome to bind to the mRNA, and synthesis to occur. Localization is just one way RNA can control platelet protein synthesis. Platelets also possess splicing machinery, and can differentially splice pre-mRNA, leading to differing protein expression. Other forms of RNA, such as micro RNA (miRNA) can also contribute to regulating protein synthesis, but the roles of circular RNA (circRNA) and other long non-coding RNAs (lncRNA) are not as well studied.

Controlling Platelet Function with RNA

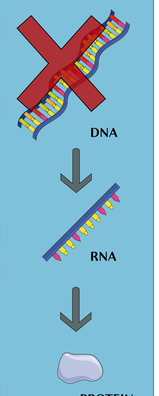
Platelets do not have a nucleus- No DNA
- Still able to synthesize proteins!
- Need to control protein synthesis through RNA

RNA-based control of protein synthesis can occur at the four phases of translation:
- INITIATION
- ELONGATION
- TERMINATION
- RIBOSOME RECYCLING

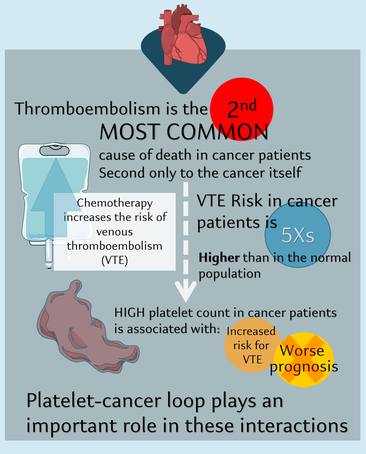
Stages of RNA translation

Control with mRNA itself or other RNA, like:
- Micro RNA (miRNA)
  - Signal for mRNA sequences to be degraded
- pre-mRNA
  - Splice variants of mRNA

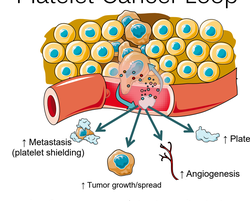
Circular RNA and long-non-coding RNA are also present but their role is less clear



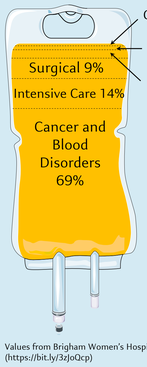
Platelets and Cancer



Platelet-Cancer Loop



1. Cancer cells release factors to recruit platelets
2. Platelets are activated and bind to cancer cells and release microparticles
3. Microparticles contain factors that promote:
   - I. Metastasis
   - II. Tumor growth
   - III. Angiogenesis
   - IV. Platelet aggregation
4. Platelets can also shield cancer cells from immune cells and allow the tumor fragments to move through the circulation undetected



Values from Brigham Women's Hospital Blood Bank (https://bit.ly/3zJoQcp)

Despite the negative role platelets often play in cancer, they frequently need to be transfused during treatment

- Chemotherapy and radiation can destroy platelets or their precursor megakaryocytes, leading to low platelet counts
- Low platelets increases the risk for bleeding and possibly treatment delays (e.g. surgery)
- Most common prophylactic treatment is platelet transfusions, despite platelet-cancer interactions
- Platelet transfusions in cancer patients are often not very effective, and can result in:
  - High rates of bleeding
  - Platelet refractoriness (unsatisfactory levels of platelets following transfusion)
  - Treatment delays
  - Complications with antithrombotic drugs (when indicated)

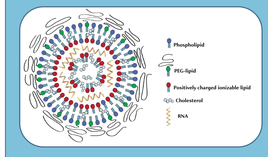
Want to modify platelets to improve their function, which could enable fewer platelets to be transfused, or modify the protein content of platelets to help prevent platelet refractoriness

PROBLEM: Platelet transfusions are a critical component of cancer treatments, but are not effective at preventing bleeding, and can increase the risk of metastasis and other complications.

GOALS: Create modified platelets that have enhanced or added function to improve platelet transfusions

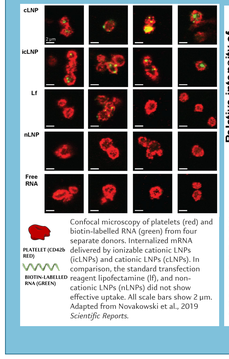
METHODS: Modify platelets by delivery of RNA through lipid nanoparticles (LNPs)

Can use Lipid Nanoparticles (LNPs) to deliver RNA to platelets



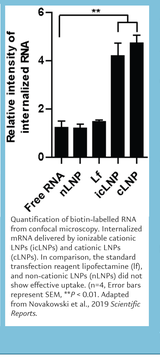
LNPs are an effective means to deliver mRNA to cells, compared to direct RNA delivery as they:
- Protect mRNA from degradation
- Minimize immune response
- Prevent rapid renal clearance

This is further shown in current COVID-19 vaccines



PLATELET (CD42b BEDS)
BIOTIN-LABELLED RNA (GREEN)

Confocal microscopy of platelets (red) and biotin-labelled RNA (green) from four separate donors. Internalized mRNA delivered by ionizable cationic LNPs (icLNPs) and cationic LNPs (cLNPs). In comparison, the standard transfection reagent lipofectamine (lf), and non-cationic LNPs (nLNPs) did not show effective uptake. All scale bars show 2 μm. Adapted from Novakowski et al., 2019 *Scientific Reports.*



Quantification of biotin-labelled RNA from confocal microscopy. Internalized mRNA delivered by ionizable cationic LNPs (icLNPs) and cationic LNPs (cLNPs). In comparison, the standard transfection reagent lipofectamine (lf), and non-cationic LNPs (nLNPs) did not show effective uptake. (n=4, Error bars represent SEM, $^{**}P < 0.01$. Adapted from Novakowski et al., 2019 *Scientific Reports.*

Proof of principle: Cationic LNPs show much higher uptake in platelets

No translation of delivered mRNA was detected

FUTURE DIRECTIONS
Optimization of LNPs and RNA in the future
Delivery of non-reporter RNA to impact platelet function

References

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