UNIVERSITY OF NAPLES FEDERICO II

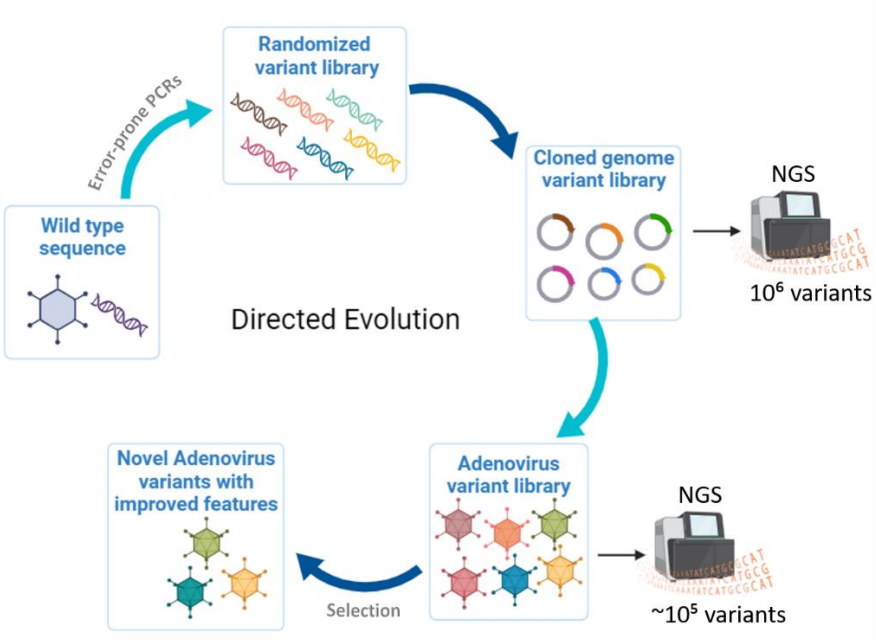
DOCTORATE IN

MOLECULAR MEDICINE AND MEDICAL BIOTECHNOLOGY

XXXVI CYCLE

Miriam Romano

Directed evolution of human Adenoviruses to improve vectored vaccines properties



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Directed evolution of human Adenoviruses to improve vectored vaccines properties

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ABSTRACT

Replication incompetent Adeno (Ad)-based vectors are able to induce potent adaptive immune responses in animal models and humans, being of interest for both infectious diseases and cancer therapy or prevention. Several Ad-based vaccines have already been approved, including those developed during the Covid-19 pandemic. Immunological potency of Ad-vectors from different serotypes can be influenced by the extent of pre-existing host immunity, different cell tropism and intracellular trafficking, also impacting the extent of innate immunity induction. Systematic approaches allowing the generation of high diversity Ad variants libraries represent invaluable tools for the selection of novel vaccine vectors with improved properties. We have set up a procedure allowing the generation of libraries of Adenoviral vectors genomic variants with an unprecedented high diversity. We successfully applied this technology to the hypervariable regions (HVR) of the hexon, the main Ad neutralization determinants also involved in intracellular trafficking of the virus and hypothesized to interact with platelet-factor 4 (PF4) contributing to pathogenesis of vaccine-induced immune thrombotic thrombocytopenia (VITT). Error prone PCR was used to introduce random mutations in single and multiple HVRs. The protocol was tailored to induce on average two aa changes within each HVR. An optimized recombineering protocol was leveraged to transfer the HVR libraries into a vector genome BAC construct and an improved rescue procedure was applied for the recovery of all available variants. Six adenoviral genome libraries were generated with mutations in single HVRs. A diversity (number of clones with different aa sequence) of ~10^5 was obtained for each library, leading overall to more than 10^6 genomic variants. This complex repertoire of Ad vectors was then successfully rescued. The vector libraries obtained were subjected to different selective pressures, leading to identification of variants enriched in different conditions, potentially associated to improved growth properties or reduced recognition by anti-Ad antibodies present in humans.

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