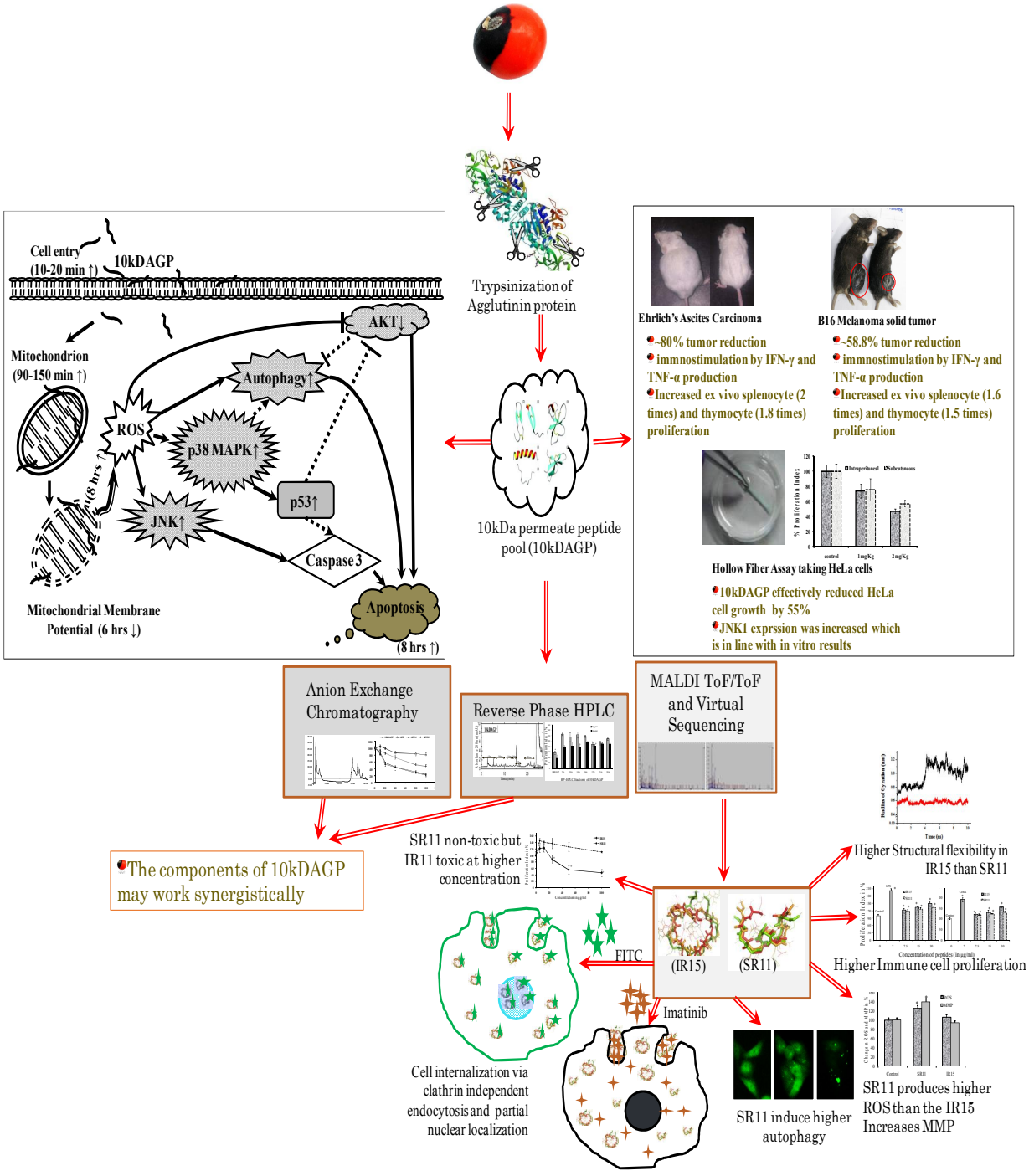
Graphical summary



ABSTRACT

Peptide pool from tryptic digest of *Abrus precatorius* agglutinin, obtained after passing 10kDa membrane cut-off (designated as 10kDAGP), was previously reported to have anticancer and immunostimulatory properties. Here, attempts have been made to study detailed mechanism of apoptosis induction by 10kDAGP mainly in human cervical cancer cells (HeLa) and to evaluate efficacy of 10kDAGP in various types of tumor models like Ehrlich's ascites (EAC), B16 melanoma solid tumor (B16M) and Hollow Fiber assay (HFA) taking HeLa cells. In addition, work has also been done to identify the peptide components of 10kDAGP to decipher function of identified peptides and also to answer whether the peptides work synergistically or individually. Results show that 10kDAGP got internalized and localized to mitochondria (90 min) in HeLa cells. It decreased mitochondrial membrane potential (MMP) starting from 6 hrs and increased ROS production which in turn led to apoptosis (8 hrs) via activating stress responsive p53, JNK1, p38 MAPK, and autophagy along with deactivating AKT. In vivo studies confirm that 10kDAGP decreases tumor volume by ~82% in EAC and 58.8% in B16M. It also increased *ex vivo* proliferation of splenocyte and thymocyte isolated from tumor bearing mice and increased TNF-α and IFN-γ in splenocyte culture supernatant. Besides this, it also decreased the viability of HeLa cells in HFA (55%). Anion exchange chromatography of 10kDAGP shows that the bound fraction is more cytotoxic than that of unbound fraction but not greater than the total 10kDAGP. Again, RP-HPLC fractions of 10kDAGP showed no toxicity comparable to 10kDAGP suggesting synergistic effect of component peptides. Furthermore, cationic (IR15) and anionic (SR11) peptides were identified from 10kDAGP by MALDI-ToF/ToF and virtual sequencing. Both peptides were synthesized. IR15 (IC_{50} = 100 µg/ml) was internalized more than SR11 (IC_{50} > 150 µg/ml) which may be due to its high structural flexibility as obtained from bioinformatic modeling. On the other hand SR11 induced higher autophagy in HeLa cells than IR15. Both the peptides facilitated internalization of Imatinib mesylate (~40%), induced mouse splenocyte and thymocyte proliferation but did not induce Nitric oxide production in RAW264.7 (mouse macrophage). In conclusion, whole 10kDAGP can be further studied as potential anticancer drug. IR15 and SR11 can be used as Cell Penetrating Peptides and SR11 can be explored further as autophagy-inducing-peptide.

Keywords: *Abrus precatorius*, apoptosis, autophagy, cell penetrating peptides, Hollow Fiber Assay, Imatinib mesylate, HeLa cell.