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Molecular pathogenesis of *Entamoeba histolytica*: Virulence genes and epigenetic gene silencing

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Recent findings

Epigenetic silencing of the expression of a gene coding for the amoebapore toxin:

Entamoeba histolytica trophozoites produce amoebapores, a family of small (77 aa) amphipathic peptides capable of insertion into eukaryotic and bacterial membranes and causing cellular lysis. Stable transfection of virulent amoebic trophozoites with a hybrid plasmid construct which contained a segment of the 5' flanking sequence of the *Ehap-a* gene caused, within a few weeks, the complete inhibition of transcription and translation of this gene. Removal of the plasmid resulted in a stable, silenced, plasmid-less trophozoite line (G3). The size of the 5' flanking element (between 470-850 bp) was crucial for the gene silencing to occur. Sequence analysis of the 470 bp 5' flanking element of the *Ehap-a* gene revealed a segment of a neighboring short, interspersed repetitive element (SINE1) which is actively transcribed in the parasite. Elimination of the SINE1 sequences from the plasmid construct prevented the silencing of the *Ehap-a* gene in new transfectants. Placing the CAT reporter gene under the control of the 5'SINE sequences enabled the transcription of CAT proving the existence of an active promoter element. Cytosine residues on the 5' flanking region of the *Ehap-a* gene of silenced trophozoites were found to be methylated at the 5' cytosine residues. Growing of the G3 silenced trophozoites in the presence of 5' AZA Cytidine or Trichostatin A did not reverse the gene silencing. Furthermore, re-transfection of the G3 trophozoites with another plasmid construct in which the *Ehap-a* gene was introduced under the promoter of a ribosomal gene, did not enable its expression. Our current hypothesis is that the trans-gene introduction of sequences homologous to the SINE1 repetitive element, triggered a Transcriptional Gene Silencing (TGS) cascade. A number of genes known to participate in the TGS mechanism have been identified in the genome database of *E. histolytica* and their expression is being investigated. Currently we are also searching for ds or siRNA molecules which may have triggered the DNA methylation of the *Ehap-a* gene promoter region.

Parasites that do not express amoebapore are virulence-attenuated: Potential for a live vaccine?

Trophozoites which do not express amoebapore were found to be incapable of killing mammalian cells or causing a liver abscess in the hamster animal model. Vaccination (i.p.) with live G3 silenced trophozoites evoked an immune response which partially protected animals from a subsequent challenge by a virulent strain. Since the amoebapore–deficient trophozoites can still cause limited inflammation and mucosal cell damage in infected Human colonic xenografts (colitis model), immunization with G3 trophozoites by oral route and its protective effects will be investigated.

Characterization of other genes involved in the modulation of parasite viulence.

Electrophoretic comparison by 2D-gels of lysates from two types of amoeba, one capable of producing liver abscesses in hamsters and the other incapable, revealed a number of spots which differed in their migration. Proteomic MS analysis of two of these spots showed that one of them is a Lim-like protein and the other a 20 kDa surface antigen. We are currently in the process of identifying and characterizing the molecular differences between the proteins of the two types of amoeba and trying to elucidate their possible role in parasite pathogenesis.

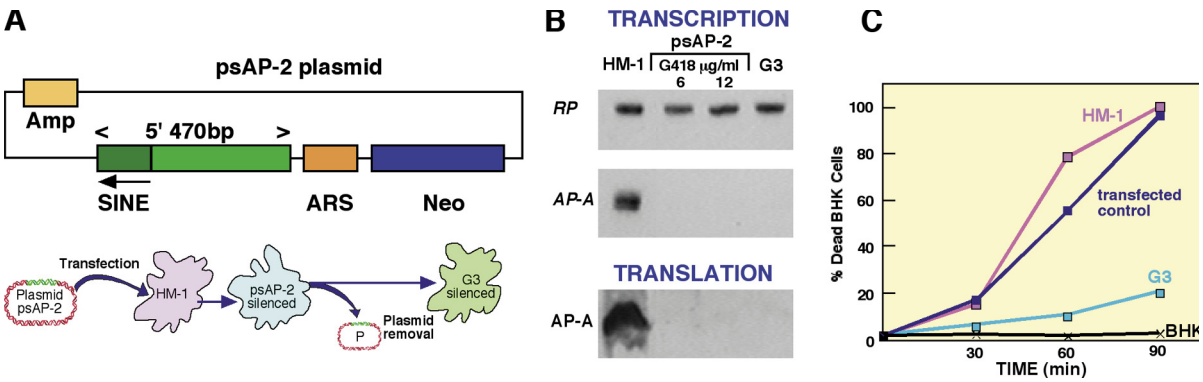

Fig. 1 *Plasmid construct and transfection events which lead to irreversible transcriptional silencing of the gene coding for amoebapore. B. Silenced trophozoites are totally devoid of Ehap-a transcript and amoebapore protein. C. Trophozoites not expressing amoebapore are not cytotoxic to mammalian cells.*

Selected publications (from 2002)

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