117° CONGRESSO NAZIONALE

della

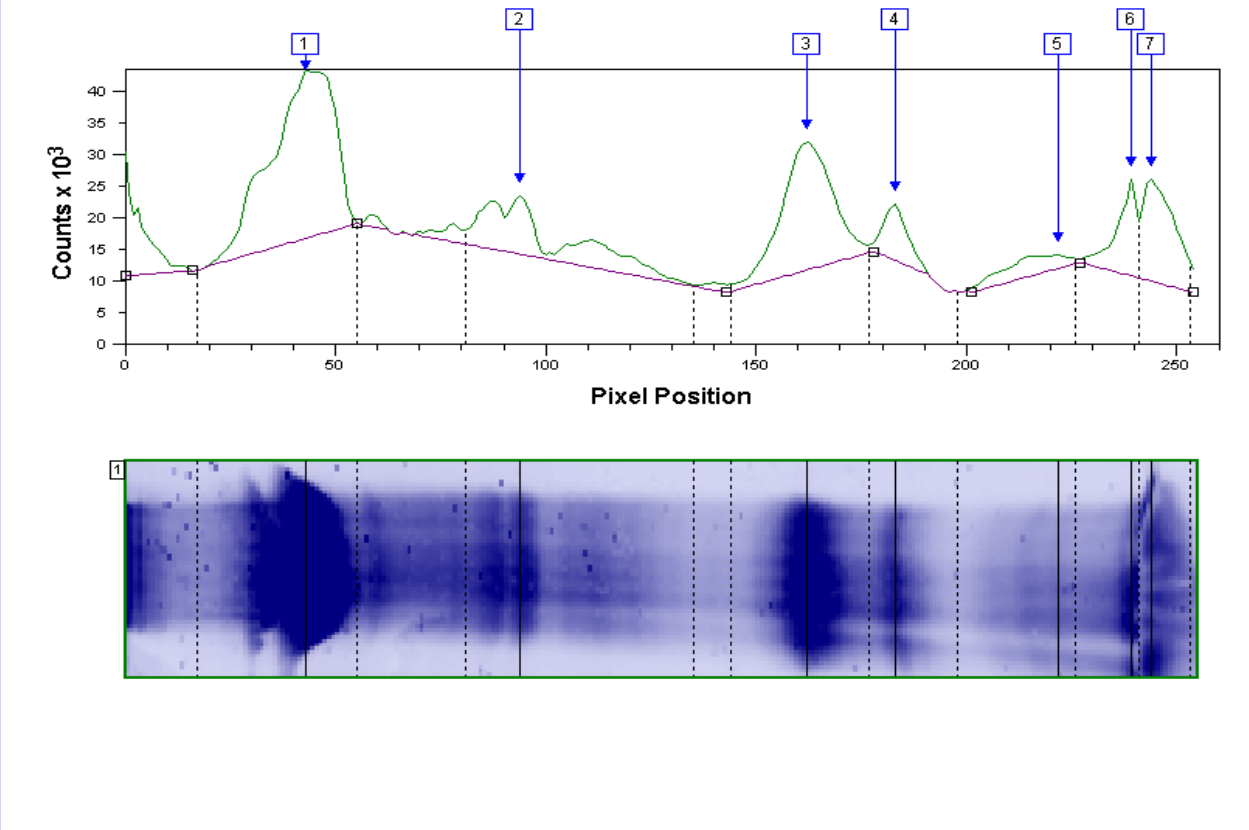
SOCIETA' ITALIANA di MEDICINA INTERNA

The Role of Differentiation Factors in Cancer and Degenerative Diseases

Pier Mario Biava, Alberto R. Frosi

Istituto di Ricovero e Cura a Carattere Scientifico Multimedica Milano

MOLECULAR WEIGHT OF DIFFERENT PROTEINS PRESENT IN ZEBRAFISH EMBRYO DURING GASTRULATION PERIOD



1) 37% molecular weight of about 97KDa
2) 14.6% molecular weight of about 45 KDa
3+4) 27.4% molecular weight of about 30-25 KDa
5) 4% molecular weight of about 20 KDa
6+7) 14% molecular weight of about 14 KDa

Table 1

List of protein identified using the nano LC-ESI-Q-TOF in Zebrafish embryo at midle-blastula-gastrula stage

Accession	Protein Name	Score	Molecular Waight	pI calculated	Sequence coverage
gi|166795887	vitellogenin 1 precursor	1108	150308	8,68	19
gi|94733730	vitellogenin 1	1039	149825	8,74	21
gi|94733733	novel protein similar to vitellogenin 1 (vg1)	913	149828	8,92	19
gi|94733734	novel protein similar to vitellogenin 1 (vg1)	835	150550	8,83	16
gi|145337918	Vtg1 protein	780	116965	9,07	18
gi|94733731	novel protein similar to vitellogenin 1 (vg1)	762	149911	8,84	19
gi|94732723	novel protein similar to vitellogenin 1 (vg1)	745	147826	8,73	17
gi|159155252*	Zgc:136383 protein	720	124413	8,78	17
gi|68448530	vitellogenin 5	559	149609	8,77	13
gi|92097636	Zgc:136383	402	28924	9,33	36
gi|63100501	Vtg1 protein	345	36580	9,23	28
gi|57864789	vitellogenin 7	341	24490	8,37	40
gi|57864783	vitellogenin 4	334	31304	9,48	27
gi|113678458	vitellogenin 2 isoform 1 precursor	323	181208	8,70	11
gi|125857991	Zgc:136383 protein	171	149328	8,93	9
gi|15209312*	procollagen type I alpha 2 chain	169	147826	9,35	4
gi|57864779	vitellogenin 2	122	69906	7,84	8
gi|11118642	vitellogenin 3 precursor	117	140477	6,92	2
gi|30327889*	vitellogenin 6	73	151677	8,84	4

Continues

Accession	Protein Name	Score	Molecular Waight	pI calculated	Sequence coverage
gi|13242157 *	egg envelope protein ZP2 variant A	71	48194	6,04	5
gi|6644111 *	nucleoside diphosphate kinase-Z1	69	17397	7,77	14
gi|18859071*	nucleoside diphosphate kinase 3	69	19558	7,68	7
gi|126632622*	novel prot. cont. a galactose binding lectin domain	67	19245	9,33	13
gi|66773080 *	mitochondrial ATP synthase beta subunit-like	66	55080	5,25	4
gi|38541767*	Ppia protein	60	19745	9,30	13
gi|1865782	HSC70 protein	58	71473	5,18	2
gi|28279108	heat shock protein 8	58	71382	5,32	4
gi|41152402*	histone H2B 3	49	13940	10,31	11
gi|41393113*	collagen, type I, alpha 1b precursor	46	137815	5,39	4
gi|94732492 *	ras homolog gene family, member F	46	24035	9,00	6
gi|47778620 *	tryptophan hydroxylase D2	45	55686	6,56	1
gi|68448517 *	zona pellucida glycoprotein 3.2 precursor	44	47365	4,92	2
gi|326677766 *	PREDICTED: RIMS-binding protein 2-like	41	138659	5,86	0
gi|112419298	Vtg3 protein	40	60622	6,32	2
gi|54400406 *	glutaredoxin 3	39	36541	5,18	11
gi|41152400*	peptidylprolyl isomerase A, like	37	17763	8,26	7

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 36 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

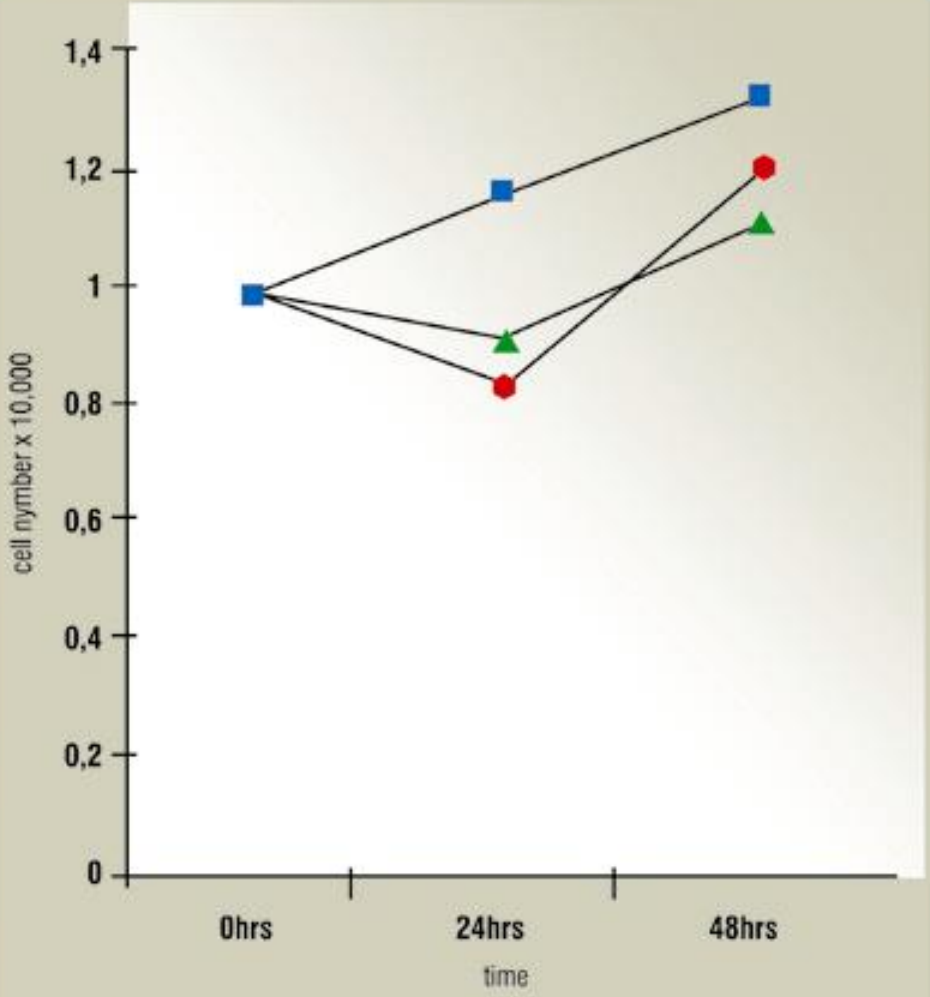
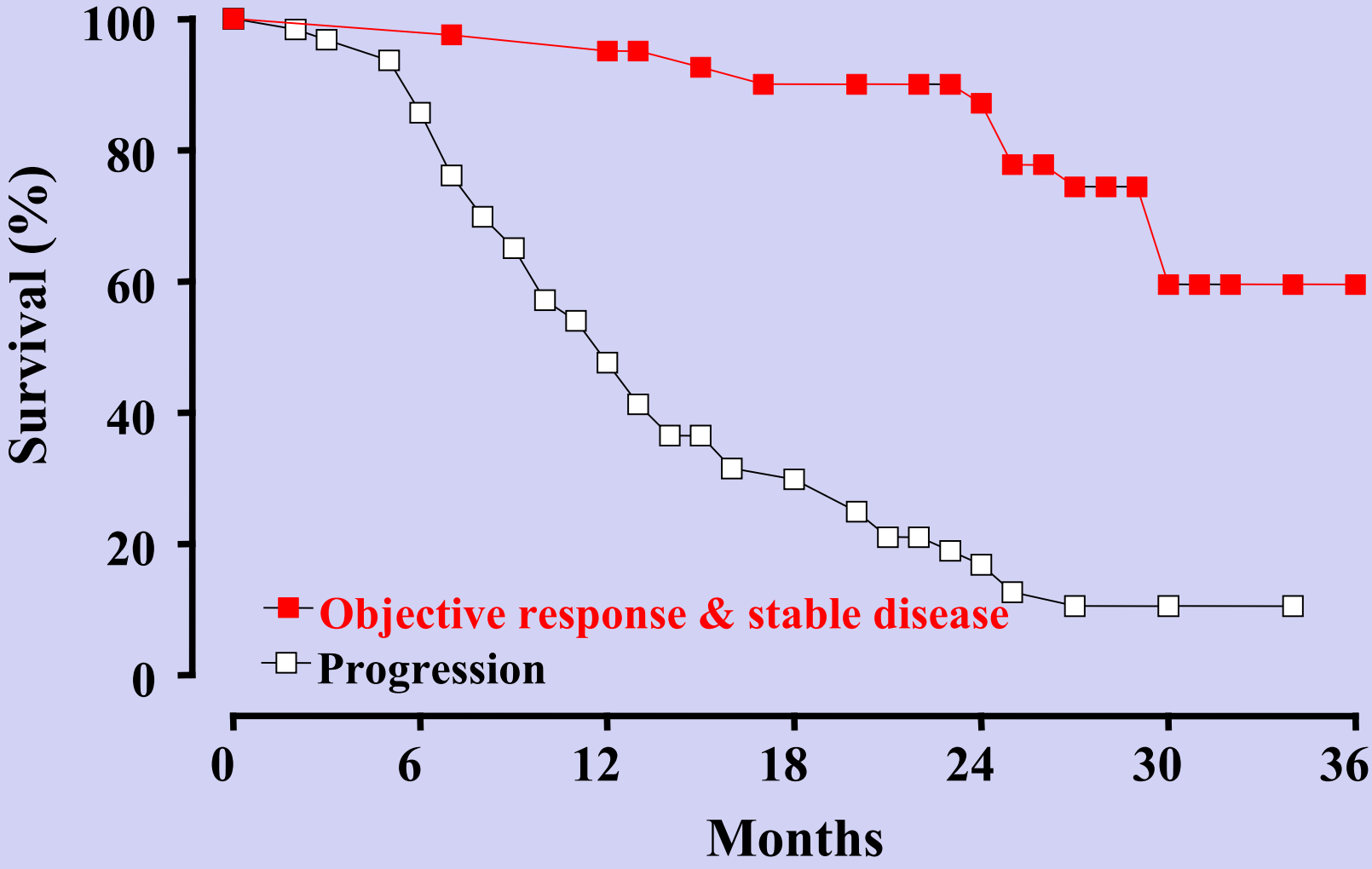


Fig.12
Cell proliferation curve of Breast carcinoma after the treatment with zebrafish embryonic proteins.

- control cells.
- stage II (5 somites) treated cells.
- stage III (20 somites) treated cells

Treatment with stem cell differentiation stage factors of intermediate-advanced HCC: an open randomized clinical trial.



Livraghi T, Meloni F, Frosi A et al. Oncol Res 2005

Stem cells – a definition

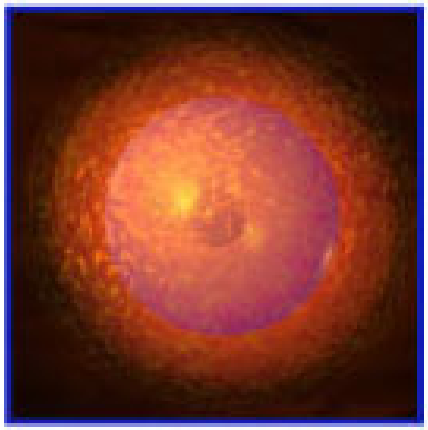
- Undifferentiated cells that give rise to all of the body's cells and organs. Three sources of stem cells: embryonic, adult and fetal. Research performed on humans and in animal models.

- Capacity for self-renewal: through repeated cell division, every stem cell can form two identical copies of itself

- … and multipotency: stem cells can form progeny that can differentiate, i.e. develop into one of the different types of cells that comprise the living organism

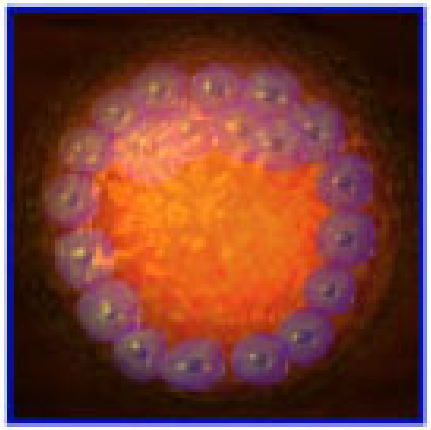
- In principle, each one of the human body's 200 different cell types can be cultured from a single immature stem cell.

- Research performed on different tissue types: Hematopoietic (blood); Neural (brain); Mesenchymal (connective tissue, muscles, blood vessels)



Single Cell Embryo

Totipotent



5-7 Day Embryo

Embryonic Stem (ES) Cells

Pluripotent

Infant

Adult

"Adult" Stem Cells

Multipotent

Cord Blood Stem Cells

Placental Stem Cells

Multipotent

Special characteristics of stem cells

- **Self-renewal** (proliferation)- the ability of a stem cell to clone itself indefinitely by cell division.

- **Asymmetric cell division – more to come**

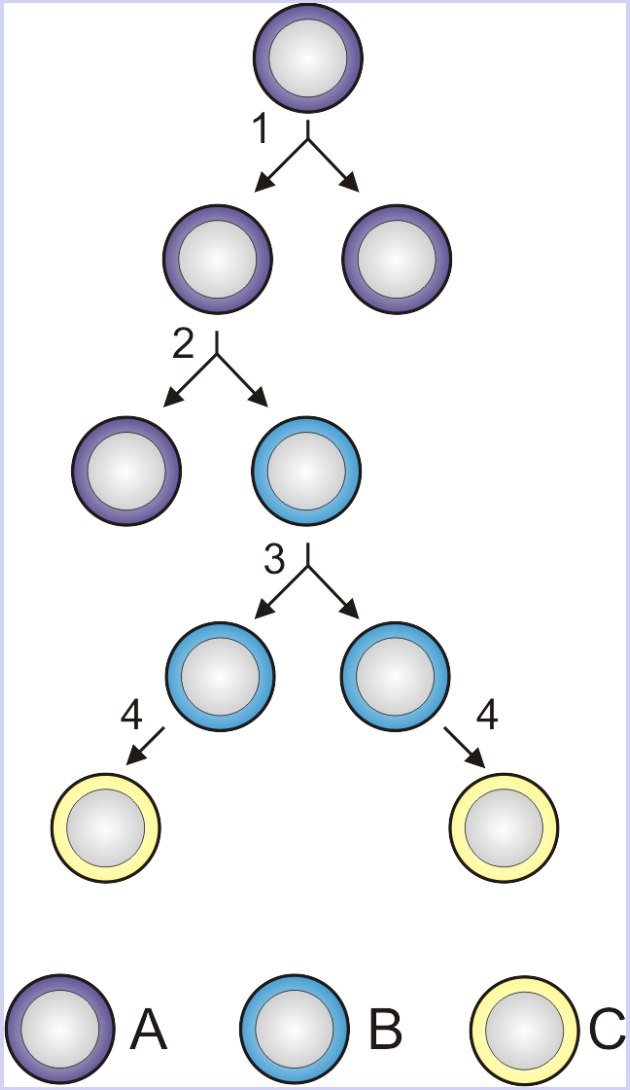
- **Relocation and Differentiation** are abilities of stem cells to “migrate” to where they’re needed in the body and specialize into a particular type of mature cell

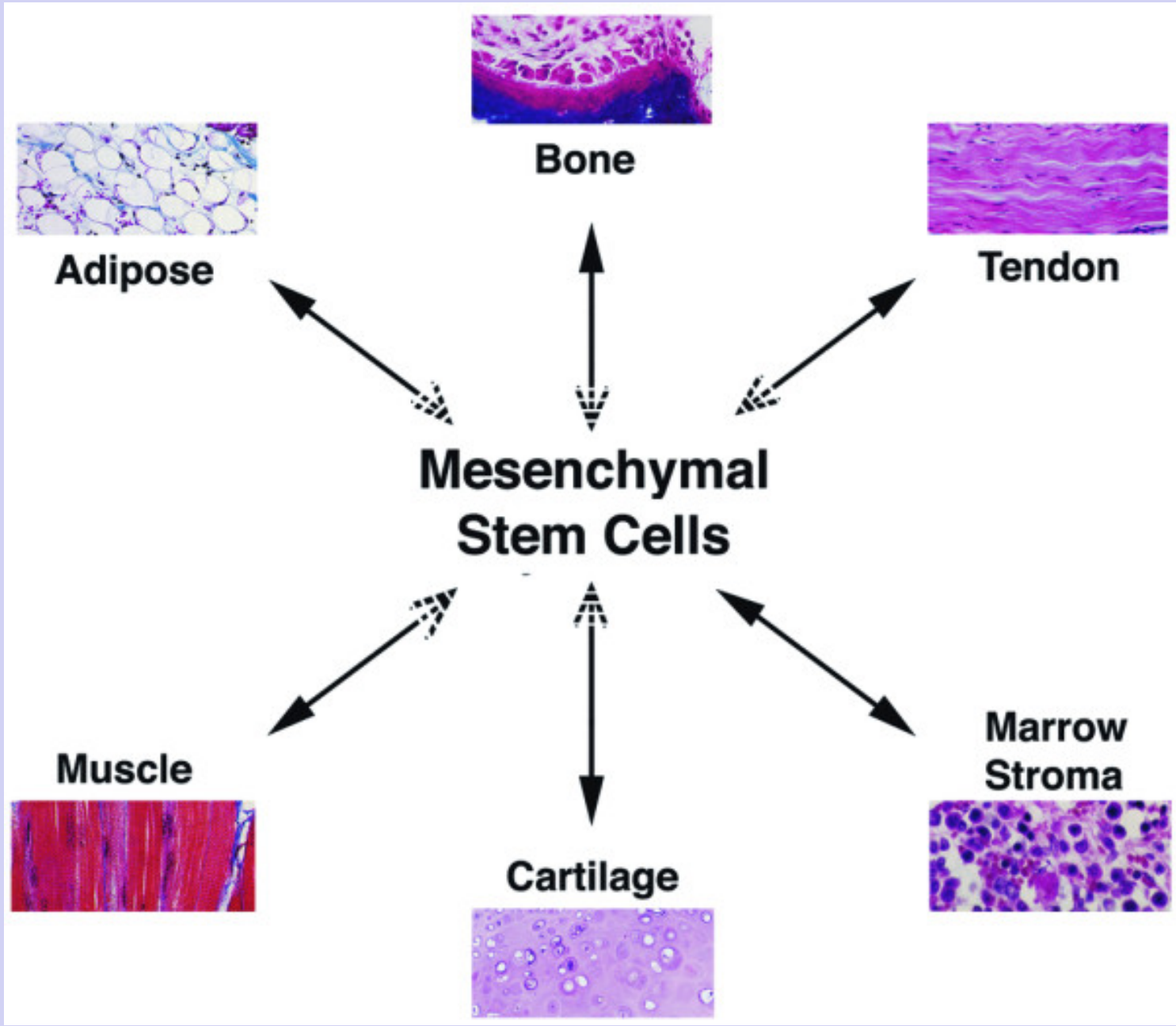
Stem cell division and differentiation

LEGEND

A - stem cell
B - progenitor cell
C - differentiated cell

1 - symmetric stem cell division
2 - asymmetric stem cell division
3 - progenitor division
4 - terminal differentiation




Bone
Adipose
Tendon
Mesenchymal
Stem Cells
Muscle
Marrow
Stroma
Cartilage

Potential to cure a variety of diseases

- **Blood cells** Cancer, immunodeficiencies, inherited blood, diseases, leukaemia
- **Bone cells** Osteoporosis
- **Cartilage cells** Osteoarthritis
- **Heart muscle cells** Heart attacks, congestive heart failure
- **Insulin-producing cells** Diabetes
- **Liver cells** Hepatitis, cirrhosis
- **Nerve cells** Stroke, Parkinson's disease, Alzheimer's disease, spinal cord injury, multiple sclerosis
- **Retinal cells** Macular degeneration
- **Skeletal muscle cells** Muscular dystrophy
- **Skin cells** Skin cells burns, wound healing

Two therapeutic approaches have been distinguished:

- Cellular therapy (i.e. cell transplantation)
- Pharmaceutical approach (signaling substances)

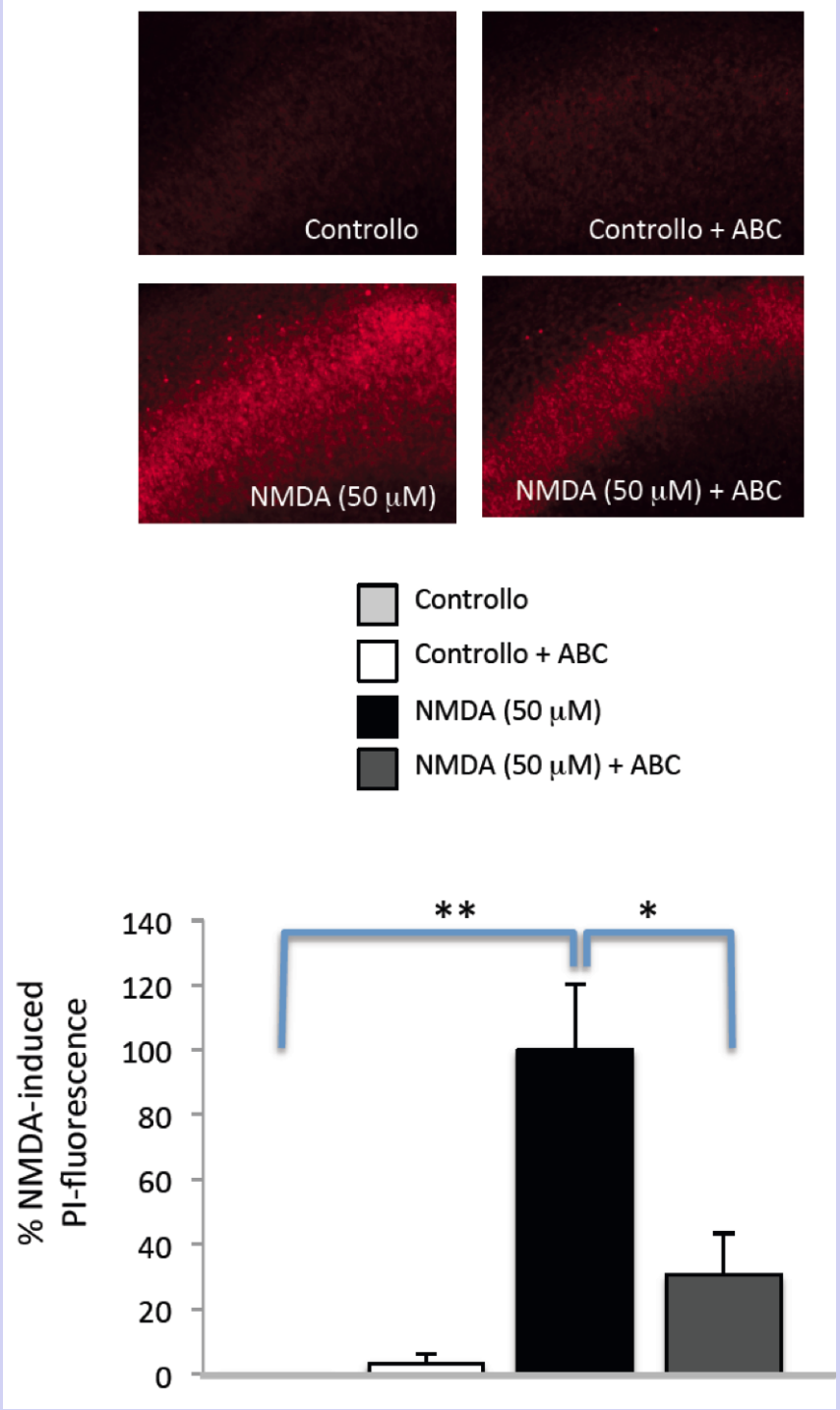


Figura 2: Immunofluorescenza con propidio ioduro dell'area CA1 dell'ippocampo 24h dopo il 1h di deprivazione da siero in presenza o assenza della miscela ABC.

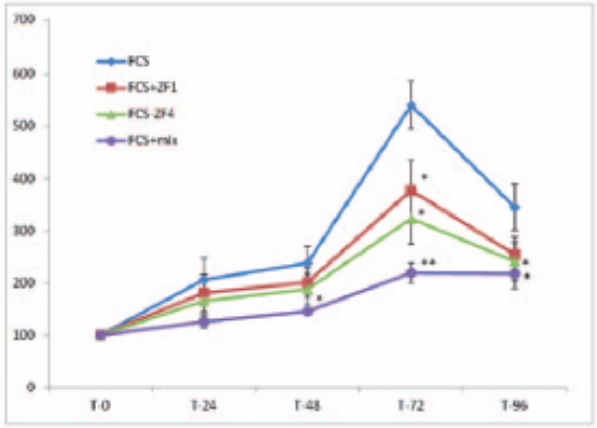


Figure 1.—Cell viability in the presence of FCS and zebrafish embryo derivative. Mean±standard error is shown; *P<0.05 *vs.* FCS; **P<0.01 *vs.* FCS.

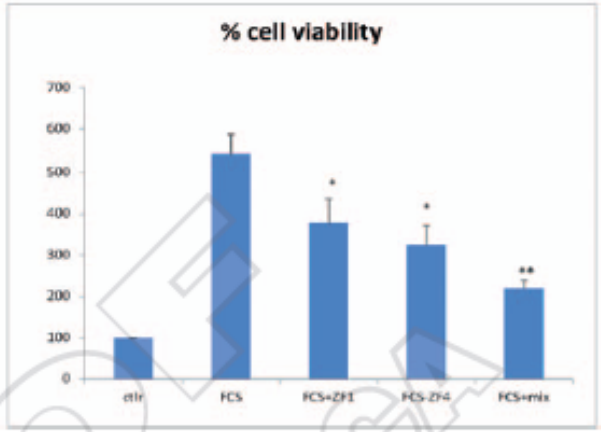


Figure 2.—Cell viability test at 72 hours following stimulation with FCS. Mean±standard error is shown; *P<0.05 *vs.* FCS; **P<0.01 *vs.* FCS.

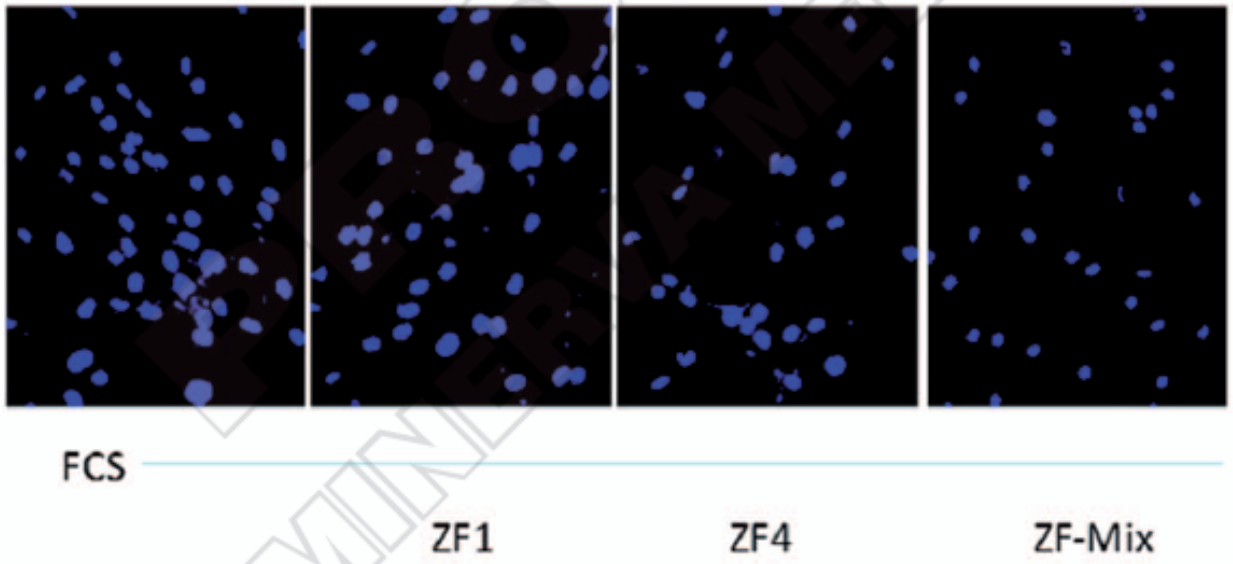
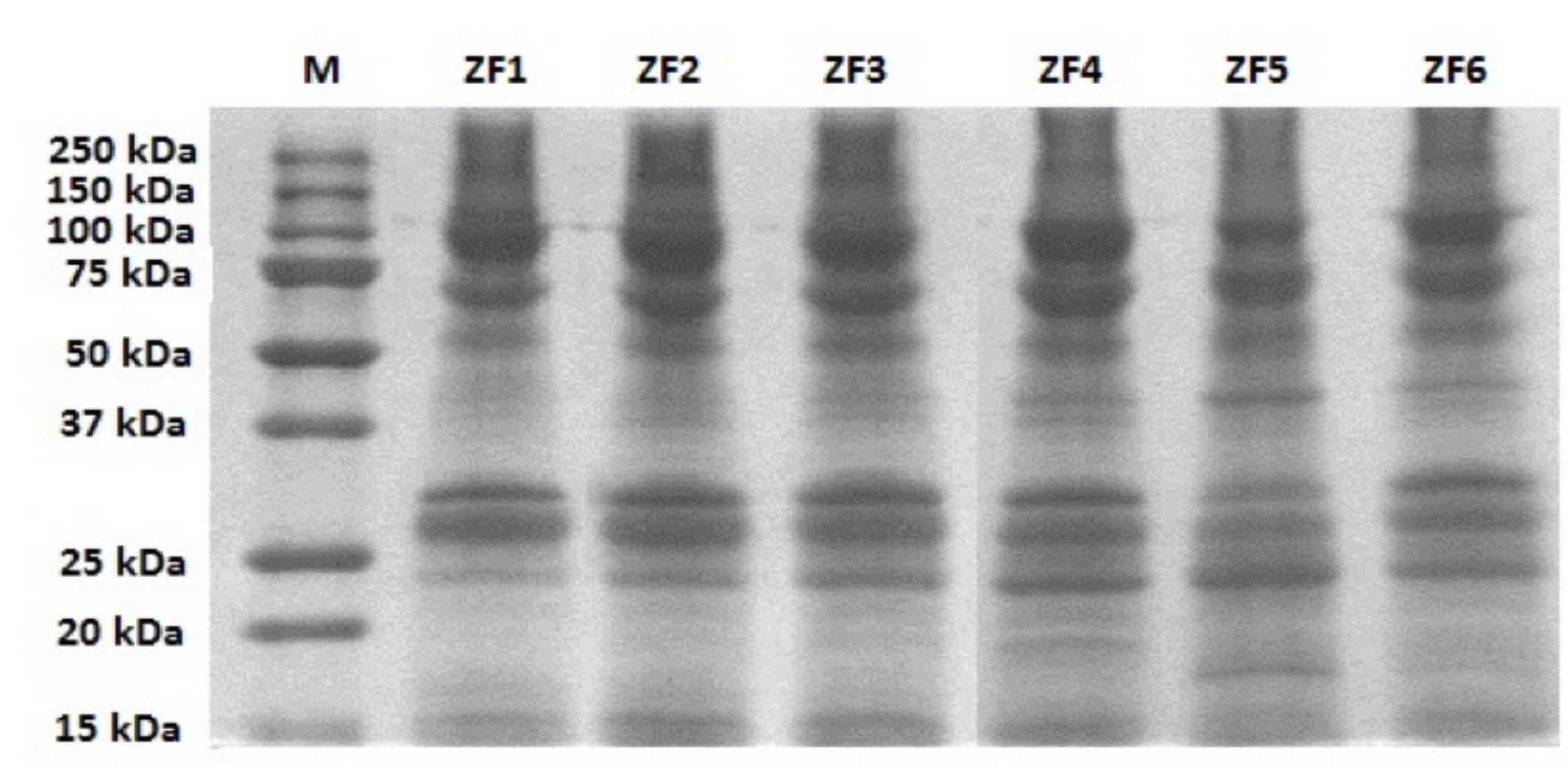


Figure 3.—Immunofluorescence through Hoestch staining.

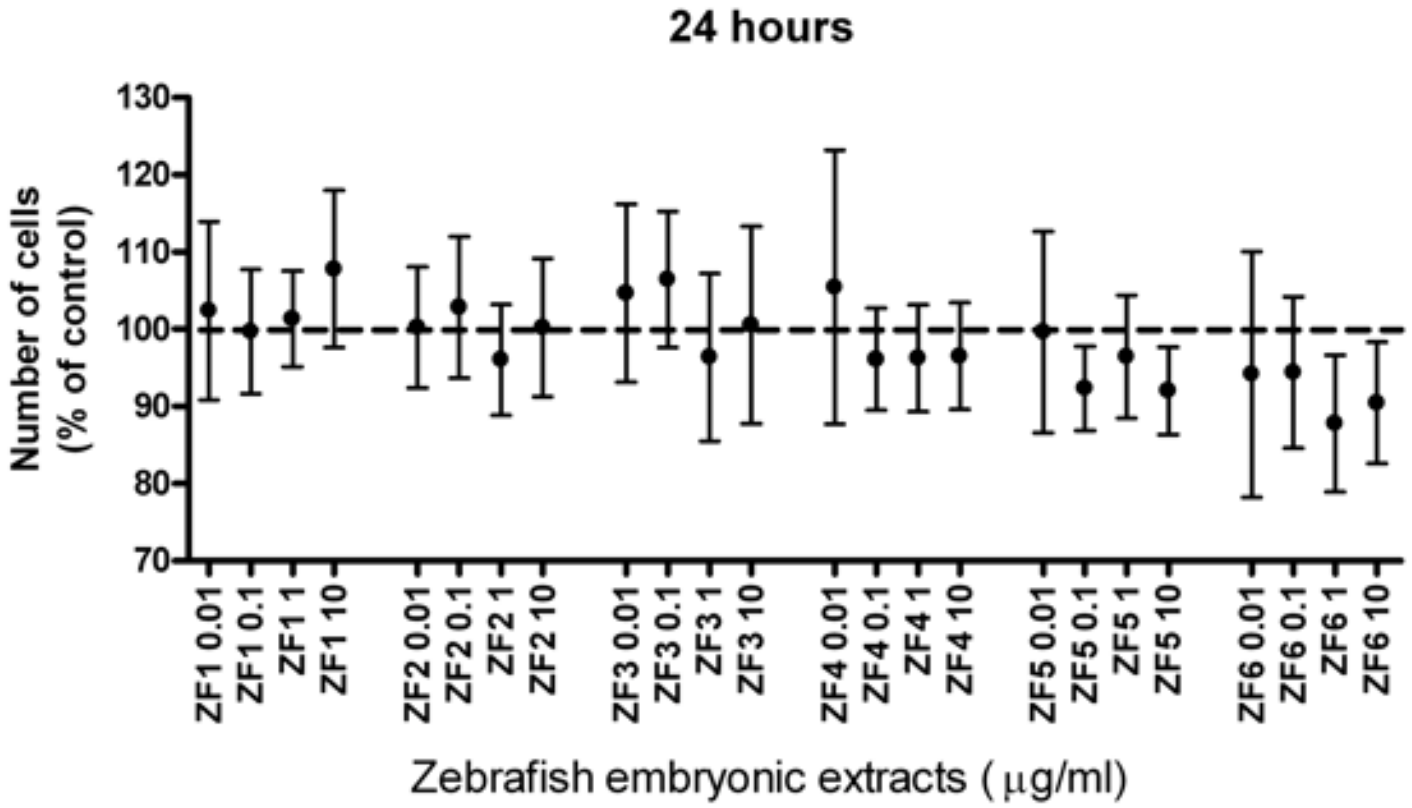
Analisi delle proteine degli estratti di zebrafish:
5 fasi di sviluppo (ZF1-ZF5) e una mix delle 5 fasi (ZF6)

SDS Page degli estratti embrionali di Zebrafish



Saggio di proliferazione:
estratti di zebrafish: 6 fasi (ZF1-ZF6) a 4 concentrazioni,
dopo 24 ore di trattamento

Saggio di proliferazione



Saggio di proliferazione:
estratti di zebrafish: 6 fasi (ZF1-ZF6) a 4 concentrazioni,
dopo 72 ore di trattamento

Saggio di proliferazione